Sugar Pills? Investigating Humphreys' Homeopathic Specifics: Utilizing Liquid Chromatography-Tandem Mass Spectrometry for an In-Depth Look at Nineteenth-Century American Homeopathy

Co-Authors:

Bronté E. Gerber

Elizabeth R. LaFave

Supervisor:

Dr. Eli G. Hvastkovs

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Abstract:

Objective: Frederick K. Humphreys founded the Humphreys' Homeopathic Medicine Company in the mid-19th century during the rise of greater acceptance and popularity of homeopathy in the United States, rivaling that of orthodox medicine. The relatively high cost and low success rate of traditional practices furthered the popularity of homeopathic medicine, despite claims of false advertising and criticisms found scattered within many patient success stories. In an effort to better understand the ingredients used in these popular homeopathic remedies, while determining the validity of Humphreys' treatments marketed by specific illness, the components of each were identified using mass spectrometry (MS) techniques.

Methods: Tandem mass spectrometry analysis (MS/MS) was used to pinpoint similarities in the ingredients of four "specifics" sold by Humphreys' company.

Results: The spectra showed similar base peak ions (*m/z 433, 381, 365, 271, 203, 185*) for each pill leading to the hypothesis that all four samples contained the same basic ingredients. Further MS/MS analyses identified these base peak ions as adduct peaks of sucrose (*m/z 365, 381*), its related ions (*m/z 433, 203, 185*), and apigenin (*m/z 271*) in each of the advertised remedies analyzed.

Discussion/Conclusions: Although these data demonstrate that Humphreys' specifics contained the same basic ingredients for each ailment, apigenin has been reported to have potential medicinal properties. When placed within a historical context, these results support both patient praise of homeopathy and provide evidence that homeopathic remedies had a contributing role in lowering mortality rates when compared to traditional medicine in the late 19th century.

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1. **Introduction:**

Homeopathy, commonly dubbed as the "philosophy of healing," entered the American medical landscape nearly two centuries ago.[1] Although the principles of homeopathy were based on Samuel Hahnemann's law of similars, defined as the use of a single medication to treat all symptoms afflicting a patient using only minimal doses, the customs continued to evolve over time.[1] Specifically, American homeopathy had been shaped by the same social, scientific, and philosophical forces as other schools of the healing art, leading to this alternative medicine to be regarded in a "friendlier" light by society.[1] As a result, the popularity of homeopathy grew exponentially during the latter portion of the 19th century, especially in middle- to upper-class families who recognized the lack of evidence for traditional medical treatments published in early medical and scientific texts.[1]

1.1. The Role of Homeopathy in the Family

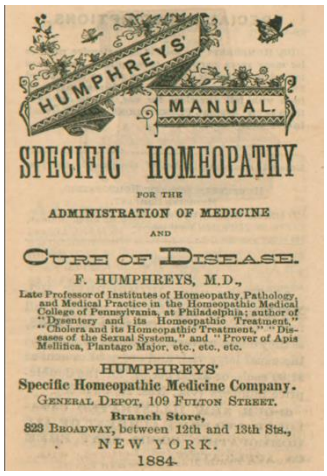
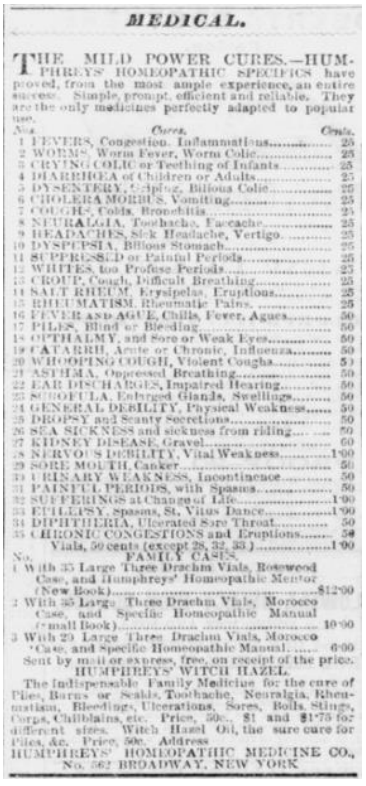

HUMPHREYS'
MANUAL.
SPECIFIC HOMEOPATHY
FOR THE
ADMINISTRATION OF MEDICINE
AND
CURE OF DISEASE.
F. HUMPHREYS, M.D.,
Late Professor of Institutes of Homeopathy, Pathology, and Medical Practice in the Homeopathic Medical College of Pennsylvania, at Philadelphia; author of "Dysentery and its Homeopathic Treatment," "Cholera and its Homeopathic Treatment," "Diseases of the Sexual System," and "Prover of Apis Mellifica, Plantago Major, etc., etc., etc.
HUMPHREYS'
Specific Homeopathic Medicine Company.
GENERAL DEPOT, 109 FULTON STREET.
Branch Store,
823 BROADWAY, between 12th and 13th Sts.,
NEW YORK.
1884.

Figure 1: Humphreys' homeopathic treatment guide marketed specifically to families in the mid-to-late 19th century, claiming upwards of one hundred thousand customers, to use as an at-home alternative to traditional allopathic practices.[3]

Practitioners of homeopathy originally chose to treat symptoms based on the totality of disease, focusing on the underlying cause to determine an appropriate course of treatment. Addressing the root of the affliction was often easier when compared to conventional allopathy.[1] Through most of the 19th century, licensed physicians, lay practitioners, and self-care advocates practiced homeopathy.[2] Contrary to the often dubbed "quacks," homeopathic specialists came from a diverse background, mirroring the standards of traditional medicine, as many homeopaths were licensed physicians or other certified medical professionals.[2] As this alternative treatment grew customary, commercial pamphlets and academic texts, as shown in Figure 1,


MEDICAL.

THE MILD POWER CURES.—HUMPHREYS' HOMEOPATHIC SPECIFICS have proved, from the most ample experience, an entire success. Simple, prompt, efficient and reliable. They are the only medicines perfectly adapted to popular use.

No.	Cures.	Cents.
1	FEVERS, Congestion, Inflammations	25
2	WORMS, Worm Fever, Worm Colic	25
3	CRYING COLIC or Teething of Infants	25
4	DIARRHŒA of Children or Adults	25
5	DYSENTERY, Griping, Bilious Colic	25
6	CHOLERA MORBUS, Vomiting	25
7	COUGHS, Colds, Bronchitis	25
8	NEURALGIA, Toothache, Faceache	25
9	HEADACHES, Sick Headache, Vertigo	25
10	DYSPEPSIA, Bilious Stomach	25
11	SUPPRESSED or Painful Periods	25
12	WHITES, too Profuse Periods	25
13	CROUP, Cough, Difficult Breathing	25
14	SALT RHEUM, Erysipelas, Eruptions	25
15	RHEUMATISM, Rheumatic Pains	25
16	FEVER AND AGUE, Chills, Fever, Agues	50
17	PILES, Blind or Bleeding	50
18	OPTHALMY, and Sore or Weak Eyes	50
19	CATARRH, Acute or Chronic, Influenza	50
20	WHOOPING COUGH, Violent Coughs	50
21	ASTHMA, Oppressed Breathing	50
22	EAR DISCHARGES, Impaired Hearing	50
23	SCROFULA, Enlarged Glands, Swellings	50
24	GENERAL DEBILITY, Physical Weakness	50
25	DROPSY and Scanty Secretions	50
26	SEA SICKNESS and sickness from riding	50
27	KIDNEY DISEASE, Gravel	50
28	NERVOUS DEBILITY, Vital Weakness	1 00
29	SORE MOUTH, Canker	50
30	URINARY WEAKNESS, Incontinence	50
31	PAINFUL PERIODS, with Spasms	50
32	SUFFERINGS at Change of Life	1 00
33	EPILLEPSY, Spasms, St. Vitus Dance	1 00
34	DIPHTHERIA, Ulcerated Sore Throat	50
35	CHRONIC CONGESTIONS and Eruptions	50
	Vials, 50 cents (except 28, 32, 35)	1 00

FAMILY CASES.

No.		
1	With 35 Large Three Drachm Vials, Rosewood Case, and Humphreys' Homeopathic Mentor (New Book)	$12 00
2	With 35 Large Three Drachm Vials, Morocco Case, and Specific Homeopathic Manual (small Book)	10 00
3	With 20 Large Three Drachm Vials, Morocco Case, and Specific Homeopathic Manual	6 00

Sent by mail or express, free, on receipt of the price.

HUMPHREYS' WITCH HAZEL OIL

The Indispensable Family Medicine for the cure of Piles, Burns or Scalds, Toothache, Neuralgia, Rheumatism, Bleedings, Ulcerations, Sores, Boils, Stings, Corns, Chilblains, etc. Price, 50c., $1 and $1 75 for different sizes. Witch Hazel Oil, the sure cure for Piles, &c. Price, 50c. Address

HUMPHREYS' HOMEOPATHIC MEDICINE CO.,
No. 562 BROADWAY, NEW YORK

Figure 2: Advertisement from *The Philadelphia Inquirer* for Humphreys' Homeopathic Specifics (1857).[5]

aided the increase in popularity. Most notably, works such as *Family Homeopathy* (1864) and *The American Family Physician: Or, Domestic Guide to Health* (1864) enlightened parents on how best to treat their children using homeopathic remedies marketed to cure both acute and chronic diseases.[3] Coupled with the arrival of commercially available homeopathic solutions, including Humphreys' Specifics, the principles behind this ideology began evolving to accommodate more scientific dogma.

1.2. Humphreys' Homeopathic Medicine Co.

Frederick K. Humphreys (1816-1900), a homeopathic physician, quickly became a titan in his field as the demand for his products grew in the northeastern United States.[4] He founded Humphreys' Homeopathic Medicine Co. in New York City in 1853, manufacturing and selling remedies for commercial use advertised to treat a multitude of ailments.[4] His specifics gained notoriety for being the first form of effective commercial homeopathic treatments – boasting an efficacy rate of five-sixths – and were featured in popular newspapers and periodicals such as *The Philadelphia Inquirer* and *The Editor & Publisher and The Journalist.*[5-6] An example of such advertising is displayed in Figure 2, with an 1857 ad released by Humphreys' Specifics in *The Philadelphia Inquirer* proclaiming their "mild power cures" – primarily marketed for their simplicity, safety, and convenience – could treat the array of listed ailments.[5] Additionally, these advertisements, and ones similarly published during the origin of these remedies, coincide with the time period of the analyzed samples.

In addition to practicing homeopathic medicine, Humphreys authored doctrines of homeopathy including "Homeopathy vs. Allopathy" in *The American Journal of Homeopathy* (1847).[7] Published internationally, Humphreys' article detailed the precise preparatory methods of each remedy aiming to treat a specific disorder – ranging from kidney disease to diphtheria – through the combination of sugar (for taste) and vegetable medicines.[7] Additionally, Humphreys rallied against the lack of individualization in allopathic practices that homeopathy was able to provide for each patient and case.[6]

1.3. Initial Support for Homeopathy

The use of homeopathy was further supported by both patient testimonies and mortality rates of the time. Homeopathic Mutual Life Insurance Company (1868) offered discounted prices

to patients of homeopathic medicine, resultant of the decreased mortality rates.[1] A study by Dr. E. M. Kellogg for the aforementioned insurance company showed that, on average, for every 10 homeopathy patients lost, 17 allopathy patients were lost to the same condition.[1] Overall, the average mortality rate for patients treated by orthodox medicine was 16.73% versus 9.74% for homeopathic treatments.[1] These promising statistical data were additionally backed by patient testimonies often published in popular journals, including *The Republican Journal*, throughout the latter part of the 19th century and into the early 20th century.[8]

1.4. Controversy Surrounds Homeopathy

Despite the once great foothold homeopathy had in American households, the appeal of the practice began to decline. Strong evidence for scientific theory arose in the early 20th century, leading to attempts of homeopathic ideology reformation based on the developing principles. The reformation was futile, however, as the demand for homeopathy continued to diminish. Homeopathy itself was ridiculed in *The Medical Follies* (1925) – not for its failure to heal, but for its faction-like principles and traditions that ultimately prevented the practice from being recognized as a legitimate medical specialty.[9] These failures resulted in fines and allegations of false advertisement in the 1920s, with Humphreys himself being criticized in 1918 and ultimately fined $25 [Judgement No. 5635, New York] for false and fraudulent advertisement, as shown in Figure 3.[10-11]

Humphreys' Pile Ointment Witch Hazel Oil (Compound).—Shipped by Humphreys' Homeopathic Medicine Co., New York City. Analysis showed the preparation to be essentially a camphor ointment on a lard base. Falsely and fraudulently advertised. Fine, $25.—[*Notice of Judgment No. 5635; issued April 29, 1918.*]

Figure 3: Fine issued against Humphreys' Homeopathic Medicine Co. for false advertising associated with their Witch Hazel Oil in 1918.[11]

Furthered by laws stemming from the creation of the United States Food and Drug Administration (FDA) in 1906, companies were faced with this harsh scrutiny, primarily those based on homeopathic principles.[1] Allopathic physicians began an onslaught of slanderous attacks against the ever-changing principles of homeopathy as the original doctrines established by Dr. Hahnemann disappeared from the practices of more modern homeopaths.[1] Utilizing this deviation to their advantage, the biomedical community refused to recognize homeopathy as a

legitimate medical specialty solely based on sect, regardless of effectiveness to heal.[1] The FDA's protective laws unintentionally aided this decline, as homeopathic companies faced accusations of false marketing when forced to provide the public with a list of ingredients for each remedy.[11] Contrarily, the earlier homeopathic remedies (i.e. Humphreys' Homeopathic Specifics used in this study) were unimpeded by this requirement, adding to the allure of investigating the original ingredients used at the height of American homeopathy.

1.5. Modern Evaluations of Homeopathy

Modern studies performed by Australia's National Health and Medical Research Council (NHMRC) oversaw a systematic review of over 2,000 articles that evaluated the effects of homeopathic treatments on a variety of disorders and found that only 225 were conducted with enough viable scientific integrity to be properly appraised. Their conclusion was that no scientific correlation existed between homeopathic remedies and the effectiveness of treatment.[12] In contrast, the Homeopathy Research Institute (HRI) concluded that homeopathic remedies are biologically effective despite their highly diluted state.[13] Even though scant scientific evidence supports homeopathic treatments, they are still prevalent worldwide.

To build upon the foundations of homeopathy, we aim to unravel its origins during the late 19th century, the era of peak popularity and usage. Here we use liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques to identify the ingredients in one of the most sought after commercially-manufactured homeopathic kits. Although the substances utilized by Humphreys in his original products predate the mandated medicinal ingredient lists, they can be both identified and quantified through unique product ion *m/z* transitions.[14-15] Implementing this science-based framework will provide context for understanding how homeopathy thrived in an era where allopathy had begun to implement scientific change in and improvements to its practices.

2. Materials and Methods

2.1. Materials

Hydrochloric acid, formic acid, acetonitrile, and methanol were from Sigma-Aldrich. Apigenin (4′,5,7-Trihydroxyflavon) and sucrose standards were also from Sigma-Aldrich.

Humphreys' Homeopathic Specifics (CDM2003.025.001) and plant standards (chamomile, mullein) were provided by the Country Doctor Museum in Bailey, North Carolina. Purified 18 MΩ deionized water was from a Siemens high-purity water system.

2.2. Sample Preparation

A pill from each remedy analyzed was diluted in an acidic solution. A SciEx 3200 triple quadrupole was used for initial MS and MS/MS analysis for comparison in positive ion mode with apigenin, sucrose and plant (chamomile, mullein) standards of equal mass of an individual homeopathic sample (pill).

2.3. LC-MS/MS Analysis

A SciEx 3200 triple quadrupole LC-MS/MS equipped with a Gemini 3μm NX-C18 110 Å LC column (50 x 2 mm) was used. Two MS detection methods were developed. First, the sucrose content in each of the four samples was assayed, and second, mass transitions consistent with apigenin were monitored. For content determination, an analysis was performed in positive ion multiple reaction monitoring (MRM) mode for two mass transitions, characterizing fragments of sodiated sucrose (*m/z 365 → 203, 365 → 185*). LC solvents were Solvent A: 0.1% Formic Acid, 95:5 DI H_2O, and Acetonitrile and Solvent B: Acetonitrile. Gradient elution took place via 100% A 0-2 mins, ramp to 75% A mins 3-5 and 25% B. The total analysis time was 5 minutes. The flow rate was 0.200 mL min^{-1} and the injection volume was 1.00 μL. For apigenin, an analysis was again performed in positive ion MRM mode for two mass transitions characterizing fragments of apigenin (*m/z 271→153, 271→91*). LC solvents were Solvent A: 0.1% Formic Acid, 95:5 DI H_2O, and Acetonitrile, Solvent B: Acetonitrile, and Solvent C: Methanol. Isocratic elution took place via 40% A, 20% B and 40% C for 5 minutes. The flow rate was 0.200 mL min^{-1} and the injection volume was 1.00 μL.

2.4. Data Analysis

Statistical analysis was performed using Excel for MAC 2019, version 16.24, and SCIEX MultiQuant Software, version 3.0. Raw chromatograms were interpreted using OriginPro 9 software. Figures were modified using Adobe Illustrator CS6.

3. Results

3.1. Mass Spectrometry Analysis

Our goal was to first ascertain whether Humphreys' marketed treatments contained similar ingredients. Each remedy was advertised to treat a different disorder, as seen in Figures 4a and 4b below (*images courtesy of the Country Doctor Museum in Bailey, NC*). It has been

Figure 4 (a): Humphreys' Homeopathic Specifics table of disorders that accompanied each kit to serve as a directive for the consumer. (b): Humphreys' Homeopathic Specifics kit containing 35 remedies each advertised to treat a different ailment.

shown previously that mass spectrometry is a powerful analytical technique used to determine the contents of solutions, specifically medications, through mass-to-charge (*m/z*) ratios and species-specific transitions.[16-26] Here we utilized electrospray ionization (ESI)-triple quadrupole tandem MS to explore base peaks in the four samples to determine if each homeopathic specific contained the same basic ingredients. When originally deciding on the samples for our analysis, we specifically chose ones that did not share any likeness in the ailments they treated. The remedies culled include: #4 Diarrhea, #17 Piles, #32 Disorders of the Heart, and #34 Diphtheria. The spectra showcasing overall *m/z* (MS1) within the range *50-500 m/z* for each sample are shown in Figure 5a-d.

Figure 5 highlights that the samples are very similar to each other, providing evidence to support that Humphreys' specifics did not have formulae unique to each remedy. Specifically, the spectra show the same base peak ions, or most prevalent ions (*m/z 433, 381, 365, 271, 203, 185*), in each sample. Essentially, these mass spectra exhibit the ionized, or charged, components within each sample sorted by *m/z*, which can be selected and further analyzed to unveil structural information based upon further *m/z* sorting.

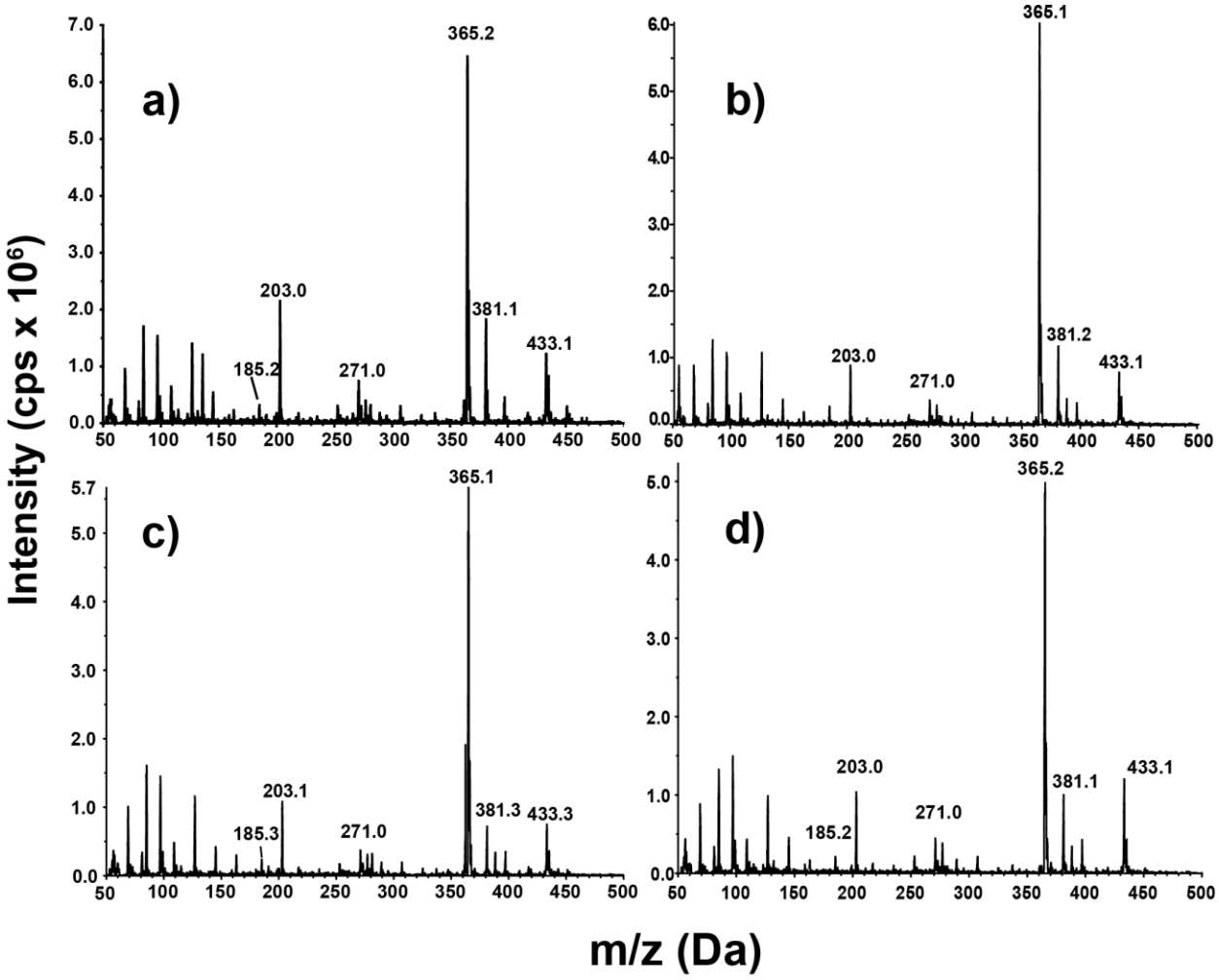


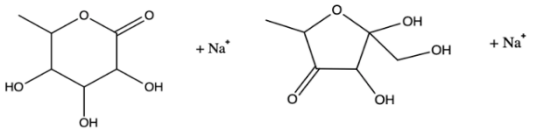
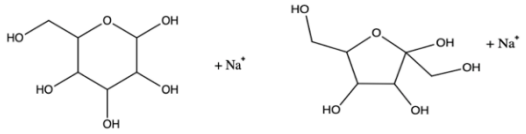
Figure 5: Mass spectra of Humphreys' Homeopathic Specifics showcasing the commonality of six primary base peaks unassociated with solvent in samples (a) #32 Disorders of the Heart, (b) #4 Diarrhea, (c) #17 Piles, and (d) #34 Diphtheria. The six primary base peak ions present in a-d were selected and further analyzed using tandem mass spectrometry techniques.

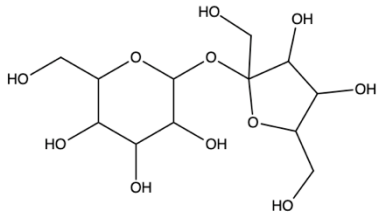
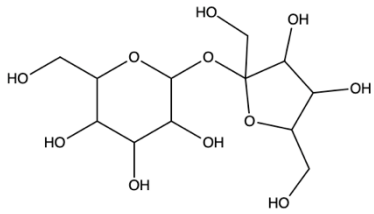
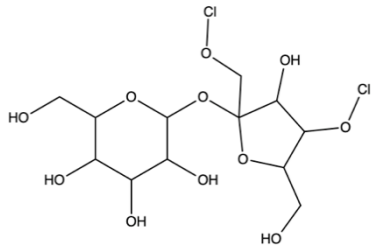
Tandem mass spectrometry (MS/MS) techniques were used to identify the components associated with six of the primary base peaks shared between the four remedies analyzed using the MS1 scan, as highlighted in Figure 5. Each base peak served as the primary parent ion when examined via Q1 MS scanning in positive ion mode, followed by product ion scanning while optimizing fragmentation parameters to obtain mass transitions for qualitative determinations of components. Through the utilization of this method, five of the six base peak ions were identified as originating from sucrose, as summarized in Table 1.[27-31]

The primary base peak ions were attributed to sodiated (*m/z 365*) and potassiated forms (*m/z 381*) of sucrose. These forms are commonly shown in positive ion mode when using ESI-MS, however, the specific interactions with glycosidic bonds in sucrose result in the sodiated

form of sucrose being the most stable and prevalent form.[32] The larger resultant compound from these two forms was also sodiated, but involved the addition of chlorine to the external hydroxyl groups. The formation of the chlorinated compounds was introduced as a result of the hydrochloric acid (HCl) used to provide an acidic environment for dissolution of the remedies.[31] Sucrose is composed of both fructose and glucose, thus when fragmented using MS/MS or digested in HCl, cleavage at glycosidic linkages can occur, resulting in the detection of individual sodiated glycosidic ions.[27-28] Each glycosidic ion can be attributed to either sodiated glucose, fructose, or a dehydrated form of either ion. Each species transition shown in Table 1 was validated using a sucrose standard equal to the mass of one Humphreys' Homeopathic Specifics pill (Figures A1-A4).

Table 1: Mass-to-charge ratios and species transitions of sucrose-related compounds identified in all four of Humphreys' Homeopathic Specifics analyzed in positive ion mode.

Mass-to-Charge (*m/z*), ESI+	Species Transitions (MS/MS)	Chemical Formula	Structure	Compound
185	n/a	Glucose: $(C_6H_{10}O_5+Na)^+$ Fructose: $(C_6H_{10}O_5+Na)^+$	 Glucose – H_2O Fructose – H_2O	Adduct formed from glycosidic bond cleavage of ion originating from $(M+Na)^+$ *m/z 365*
203	*m/z 203* → *57* (100), *127* (60), *85* (40), *83* (30), *129* (30)	Glucose: $(C_6H_{12}O_6+Na)^+$ Fructose: $(C_6H_{12}O_6+Na)^+$	 Glucose Fructose	Sodiated adduct formed from glycosidic bond cleavage of ion originating from $(M+Na)^+$ *m/z 365*

365	*m/z 365* → *203* (100), *185* (45)	$(C_{12}H_{22}O_{11}+Na)^+$		(Sucrose + $Na)^+$
381	*m/z 381* → *99* (100), *111* (60), *57* (30)	$(C_{12}H_{22}O_{11}+K)^+$		(Sucrose + $K)^+$
433	*m/z 433* → *253* (100), *277* (75), *115* (60)	$(C_{12}H_{20}Cl_2O_{11}+Na)^+$		(Sucrose + Na^+) + 2 Cl^- adducts

The final base peak ion, *m/z 271*, was identified using similar methodology. Remarkably, this ion was the only base peak ion identified that did not have a species transition corresponding to sucrose. The overall product ion spectra comparing the MS/MS for *m/z 271* from one of Humphreys' treatments (#17 Piles), a sucrose standard, and the apigenin standard are illustrated in Figures 6a-c, respectively. The resultant *m/z 271 → 153, 91, 69* species transitions were consistent with apigenin in the assortment of Humphreys' samples, as demonstrated by the match between the sample spectrum in Figure 6a and the standard shown in Figure 6c.[33] The isolation and fragmentation of *m/z 271* from the sucrose standard (Figure 6b) reveals no similarities to the apigenin standard or Humphrey's sample analyses. The Figure 6c inlay displays the likely fragmentation locations for the apigenin compound upon MS/MS analysis resulting in the most intense *m/z 153* and secondary *m/z 91* peaks. Figure A6 features the likely products that form upon fragmentation resulting in the apigenin *m/z 271* MS/MS spectrum.

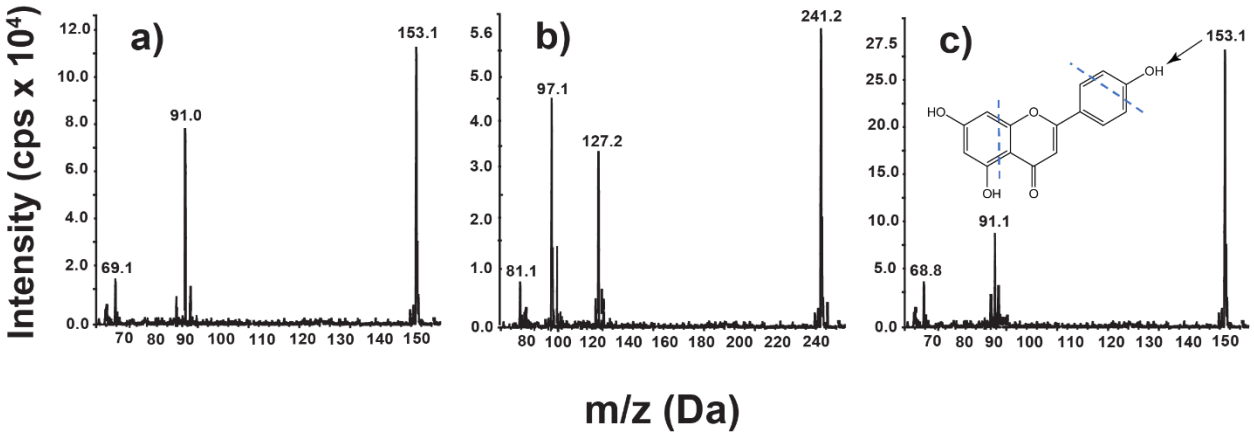


Figure 6: MS/MS of *m/z 271* detailing the determination of *m/z* in Humphreys' Homeopathic Specifics as apigenin through comparison of (a) #17 Piles, (b) Sucrose standard, and (c) Apigenin standard of equal mass to the homeopathic pill. (c) includes the structure of apigenin with the location of fragmentation corresponding to the base peak ion formed upon MS/MS analysis of apigenin.

Based on the popularity of flavonoids (i.e., chamomile), a common source of apigenin, in 19th century alternative treatments and when further coupled with the relative ubiquitous nature of sucrose, apigenin can be considered the active ingredient of Humphreys' samples.[34-37] As previously mentioned, flavonoids were largely found in period alternative forms of treatment, thus we considered two widespread sources of apigenin—chamomile and mullein. Furthermore, Humphreys' later listed chamomile as an ingredient in his remedies following the establishment of the FDA and subsequent labeling mandates in the late 20th century.[38] To distinguish between the two selected sources, and in an effort to determine the vegetable source of apigenin used in Humphreys' remedies, we applied the same MS/MS techniques previously described. The *m/z 271* species was present in both chamomile (Figure A5) and mullein (Figure A7) samples. Mullein showed a significantly more intense *m/z 271* peak compared to the most intense ion in the chamomile standard. However, the chamomile sample predominantly exhibited *m/z 284*, which is inconsistent with apigenin. Upon comparison to our Humphreys' Specifics MS1 spectra (Figure 5), no *m/z 284* is present in appreciable quantity, which points to very little sodiated apigenin in our samples. Based on this analysis, we propose that the source of the apigenin is more likely mullein than chamomile.

3.2. Liquid Chromatography-Mass Spectrometry Analysis

To quantify both apigenin and sucrose in each of Humphreys' Homeopathic Specifics, LC-MS/MS analyses were performed using positive ion MRM mode for the two most prevalent species transitions of apigenin and sucrose, *m/z 271 → 153, 91* and *m/z 365 → 203, 185,* respectively. Using linear regression analyses of both apigenin and sucrose (Figure A8), the average percent mass of the ingredients was determined for each remedy, summarized in Table 2. The table shows that the average mass of sucrose in the pills was determined to be 64.27 ± 6.25% (g/g), two orders of magnitude larger than the mass percent (g/g) of apigenin, which was determined to be 0.850 ± 0.05% (g/g).

In addition to determining the mass averages of the compounds in the pills, we also evaluated the manufacturing precision of each remedy. Here, we compared the intra-pill and inter-pill sucrose and apigenin percentage differences, also summarized in Table 2.

Table 2: LC-MS/MS of sucrose and apigenin among all four of Humphreys' remedies analyzed to determine the manufacturing precision of each individual pill (n=3).

Sample Type	Average % Mass Apigenin (g/g)	Average % Mass Sucrose (g/g)
#4 Diarrhea	0.789 ± 0.002%	52.24 ± 5.31%
#17 Piles	0.830 ± 0.002%	58.55 ± 5.67%
#32 Diseases of the Heart	0.903 ± 0.005%	71.48 ± 3.82%
#34 Diphtheria	0. 876 ± 0.007%	74.65 ± 10.21%

This average difference, when considering factors within each sample type and amongst the different remedies, showcased an approximate 20% spread. Upon combination of these data with the MS/MS analyses that identified two primary ingredients in each sample, we hypothesize that the crude methods of 19th century manufacturing presumably resulted in the low precision, as each of the remedies are not specific to a disorder in terms of utilized components.

The gap in percent (g/g) recovery range can most likely be attributed to the presence of additional filler compounds, possibly metalloid-based compounds, which were not shown in initial MS analyses due to detection limitations of the instrument used for this analytical technique. However, they can be quantified and better studied using additional mass spectrometry techniques.[39]

4. Conclusions

4.1. Summary of Results

Frederick K. Humphreys utilized homeopathic practices to establish his namesake medicinal company. The principles of homeopathy in the United States likewise applied the theory of dilution to patients, only prescribing small amounts of medicine to treat their condition, originating from the pioneer teachings of Dr. Samuel Hahnemann.[1] To assess the claims of both homeopathic physicians and patients of the late 19th century, MS/MS analysis was used to determine the ingredients of four separate Humphreys' Homeopathic Specifics.

Sucrose and apigenin were identified as the primary components in Humphreys' specifics through the utilization of MS/MS techniques. These findings are further highlighted by Humphreys' article "Homeopathy vs. Allopathy" published in *The American Journal of Homeopathy* in 1847. Although his primary goal in writing the article was to inform the American public of the success of homeopathy in comparison to traditional medicine, Humphreys did use his own company as an example treatment standard.[7] By doing so, he outlined the preparatory steps used by his company to manufacture the remedies. Specifically, Humphreys identified the use of sugar to prepare the remedies as "not to offend taste" and the harvesting of vegetable medicines during times of bloom.[7] This account establishes that sucrose was added to each remedy solely for the benefits of consumption, not medicinal value, while apigenin would be classified as an active ingredient in terms of Humphreys' vegetable medicine component. To account for the absence of percent (g/g) when combining sucrose and apigenin, we predict that additional filler ingredients were included in the overall mixture of the specifics. This hypothesis was formed based on the variety of ingredients listed by Humphreys in his later remedies marketed in the early 20th century following the establishment of the FDA.[38] Other metallic-based ingredients popularly used by Native Americans in which Humphreys' drew inspiration from, such as calcarea carbonica ($CaCO_3$), were included in his later remedies, but cannot be easily identified using our previously detailed analytical technique.[34,38]

4.2. Modern comparisons

Although these data do demonstrate that Humphreys' specifics contained the same basic ingredients for varying disorders, apigenin can be linked to widespread medicinal value during

Humphreys' time period. This is a notable determination, outlying itself from the copious amounts of sucrose added by Humphreys for taste, since sources of apigenin were used to treat all four of the ailments advertised by the samples analyzed. Dating back to early Native American and European folk medicines, sources of apigenin, primarily flavonoids, were used to treat conditions similar to those found in this research.[34] Modern studies have reinforced the use of apigenin in homeopathy, revealing anti-inflammatory, anti-bacterial, and anti-viral qualities.[40] Shao et al. explored the benefits of apigenin as an anti-cancer therapy, concluding the data showed promise of its use.[41] Of note, modern doses of apigenin are approximately 5 mg day^{-1} whereas the dose of apigenin recommended by Humphreys in his homeopathic treatment guide was approximately 0.016 mg apigenin day^{-1} (6-12 tablets per dose at 4x per day).[3,42] Humphreys did note that patients with more chronic illnesses could continuously take the specifics until their symptoms improved.[3]

To determine the apigenin source used in the samples, we analyzed two plants known for their high concentrations of apigenin — mullein and chamomile. Although both mullein and chamomile could be easily grown in the hardiness zones 3-9, which encompass the location of Humphreys' company, mullein, specifically, has an affinity to grow easily and rapidly, and is often considered to be a nuisance.[43-45] Upon these considerations, when coupled with the abundance of *m/z 271* in mullein compared to the decreased intensity of this identifying ion in chamomile, we concluded that the apigenin used in Humphreys' remedies was most likely sourced from a mullein plant.

4.3. Discussion

Humphreys' Homeopathic Medicine Co. occupied a large sector of homeopathic medicine, selling remedies directly to the consumer. However, until the establishment of the FDA, medicine companies such as Humphreys' were neither mandated nor required to list ingredients on their labels. Therefore, this burgeoning area of research, where the origins of popular homeopathy can be better understood in the United States, can be applied to track the evolution of this alternative form of treatment. The resultant analytical results can then be applied to known historical data, such as patient testimonies, to offer a more comprehensive analysis of the effectiveness and possible validity of these treatments from a scientific standpoint. Through the application of this dual approach to understanding a historical question,

the analytical techniques used in this study can be applied and expanded to further investigate the possibility of filler ingredients utilized by Humphreys in his remedies, and by both the competitors of Humphreys and those that marketed their medications solely to homeopathic physicians. Based on our research showcasing the validity of Humphreys' marketing as well as the use of mass spectrometry techniques to effectively analyze and identify these samples, future studies will offer a comparison between companies marketing to consumers and those to physicians to more fully address the question of validity during the time of peak homeopathy popularity.

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APPENDIX

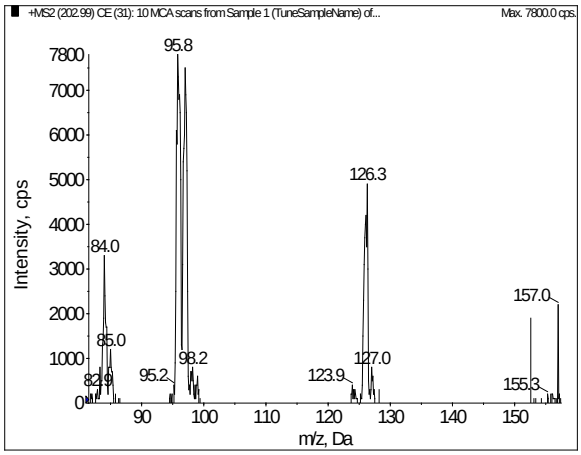

Figure A1. ESI+ product ion spectrum of sucrose M+H *m/z 203.*

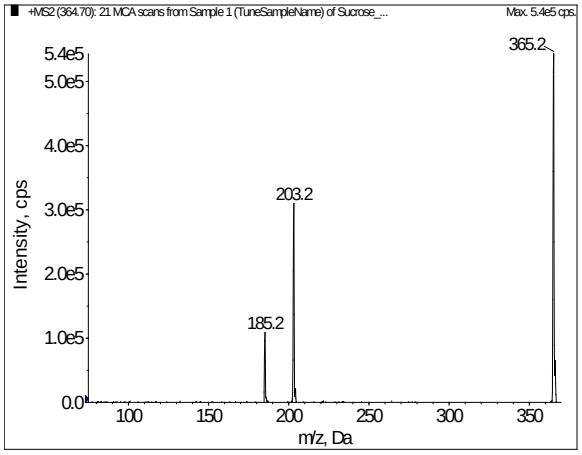

Figure A2. ESI+ product ion spectrum of sucrose M+H *m/z 365.*

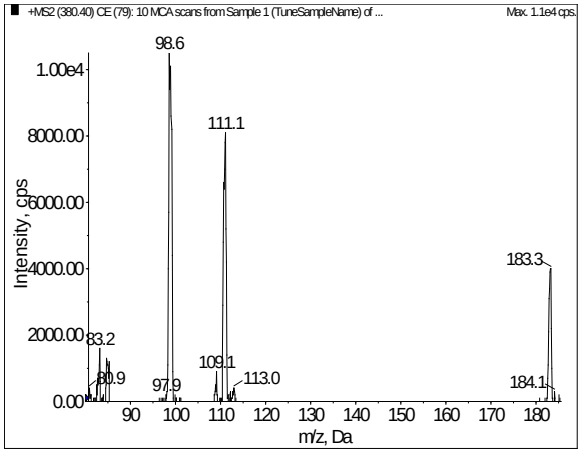

Figure A3. ESI+ product ion spectrum of sucrose M+H *m/z 381.*

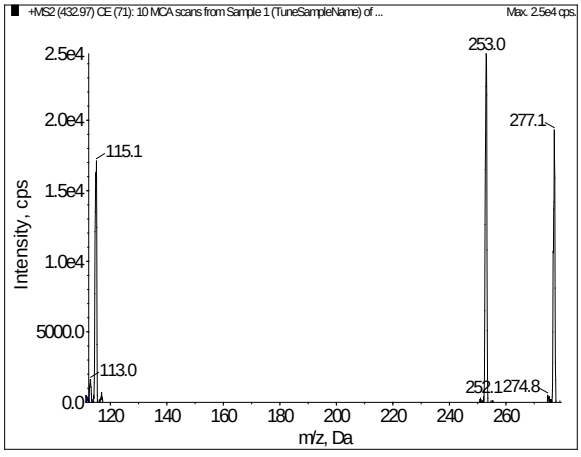

Figure A4. ESI+ product ion spectrum of sucrose M+H *m/z 433.*

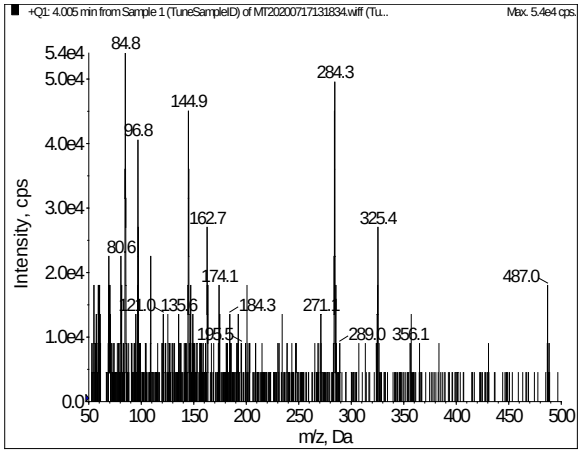

Figure A5. ESI+ MS1 spectrum of chamomilla.

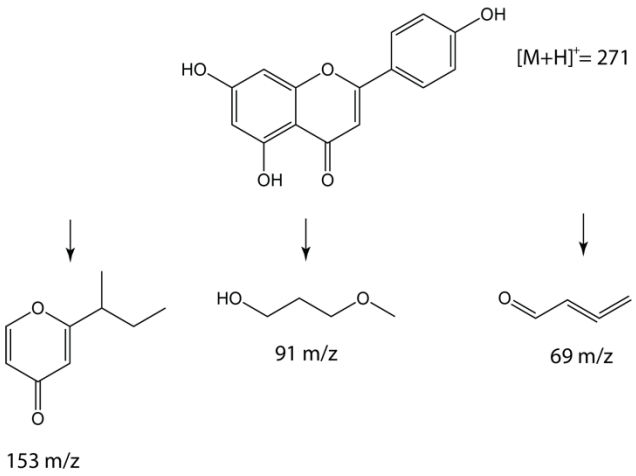

Figure A6: Fragmentation pattern of apigenin upon MS/MS analysis. *m/z* 153 was used for further LC-MS/MS analyses.

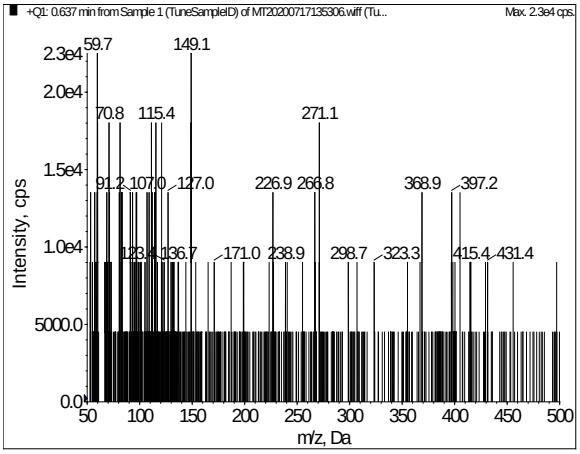

Figure A7. ESI+ MS1 spectrum of mullein.

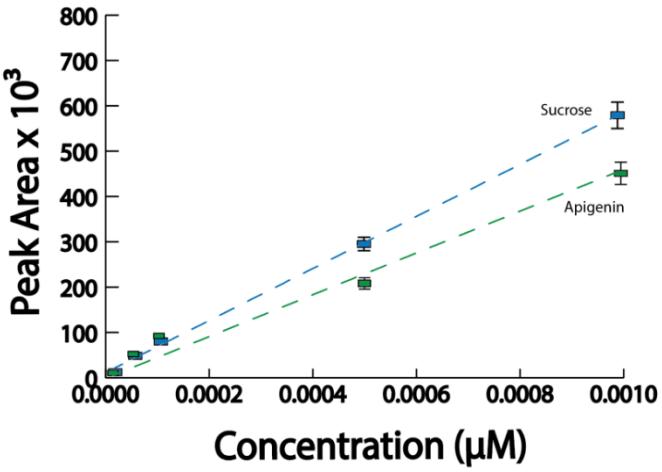

Figure A8. Standard response based on detection of M+H product ion transitions generated for sucrose (*m/z 365 → 203)* and apigenin (*m/z 271 → 153)*. Dashed lines represent least squares analysis data fits. Error bars from lower concentration points are omitted for clarity but were approximately the same as those plotted.