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Quantitative Analysis of Trimethobenzamide Hydrochloride by Ion-Pair Column Chromatography and Semiquantitative Analysis of 3,4,5-Trimethoxybenzoic Acid by Thin-Layer Chromatography

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Abstract □ An ion-pair column chromatographic/UV spectrophotometric method for assaying trimethobenzamide hydrochloride in capsules and injections is presented, as well as a method for the detection of 3,4,5-trimethoxybenzoic acid in trimethobenzamide hydrochloride bulk drug and dosage forms. Results obtained by the USP XX, Pharmacopeial Forum, and ion-pair column assay procedures are compared, and results of a collaborative study of the proposed assay and impurity detection methods are presented.

Keyphrases □ Trimethobenzamide hydrochloride—quantitative analysis by ion-pair column chromatography, semiquantitative analysis of 3,4,5-trimethoxybenzoic acid □ 3,4,5-Trimethoxybenzoic acid—semiquantitative analysis by thin-layer chromatography, quantitative analysis of trimethobenzamide hydrochloride □ Ion-pair column chromatography—quantitative analysis of trimethobenzamide hydrochloride, semiquantitative analysis of 3,4,5-trimethoxybenzoic acid by thin-layer chromatography

Monographs (1, 2) for trimethobenzamide hydrochloride (I) in capsules and injections have several shortcomings. The USP (1) assay method for the capsules involves direct dilution in 0.1 M HCl and UV spectrometric comparison with the USP reference standard. This procedure does not separate possible impurities, such as 3,4,5-trimethoxybenzoic acid (II), or interfering excipients. The *Pharmacopeial Forum* (PF) assay for injection preparations (2) also involves a UV assay which has several shortcomings.

This paper describes an ion-pair column chromatographic assay procedure, in which I is quantitatively removed from an aqueous acidic chloride column with a chlorinated organic solvent. Ether is used to remove phenolic ingredients and breakdown products prior to the elution of I.

Also, a TLC procedure is reported for the detection of II in amounts as low as 0.25% of the weight of I. Compound II is both a synthetic precursor and a breakdown product of I and could be encountered as a contaminant in drug preparations.

Table I—Recovery Data for I using the Proposed Ion-Pair, USP, and PF Injection Assay Methods

	Column Ion Pair	USP Injection	PF Injection
I, mg	8	200	200
Number of assays	10	10	10
Mean amount recovered, %	99.4	96.6	97.6
Range, %	98.3–101.0	95.4–98.1	96.1–98.4
SD	0.81	0.91	0.68
CV, %	0.82	0.94	0.70

EXPERIMENTAL

Reagents—Trimethobenzamide hydrochloride USP reference standard[1] was dried at 105°C for 4 h prior to use. Methylene chloride[2], pentane[2], and ether[2] were commercial distilled-in-glass grade. Compounds I[3] and II[3], chromatographic diatomaceous earth[4], glass wool, and the other reagents were used as received.

Apparatus—An ultrasonic bath, chromatographic tubes[5], a tamping rod[5], commercial TLC plates coated with a 250-μm layer of silica gel with a fluorescent indicator, a suitable TLC developing chamber, and a recording UV spectrophotometer were used.

Standard Preparation—Approximately 10 mg of trimethobenzamide hydrochloride USP reference standard was accurately weighed and transferred to a 100-mL volumetric flask. Methylene chloride (70 mL) was added, and the mixture was sonicated. The resulting solution was diluted to volume with methylene chloride. A 20-μg/mL solution was obtained by diluting quantitatively and stepwise with methylene chloride.

Chromatographic Column—A pledget of fine glass wool was packed in the base of a chromatographic column. A flexible spatula was used to mix 1 g of chromatographic diatomaceous earth with 500-μL of 1 M HCl in a 50-mL beaker. The mixture was transferred to a column and tamped.

Capsule Assay Preparation—The contents of ≥20 capsules were transferred to a tared container, and the average weight/capsule was determined. The

[1] USP Reference Standards; U.S. Pharmacopeial Convention, Rockville, Md.
[2] Burdick and Jackson Laboratories, Muskegon, Mich.
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Table II—Linearity of Recoveries for I using Ion-Pair and PF Injection Assay Methods

	Column Ion Pair		PF	
	I, mg	Amount Recovered, %	I, mg	Amount Recovered, %
	4.0	98.1	122	97.3
	6.0	97.8	162	98.1
	8.0	98.6	203	97.5
	10.0	98.3	243	97.5
	12.0	99.0	284	98.2
Mean (n = 15)		98.4		97.7
Range	6.0-12.0	96.2-99.4	122-162	97.0-98.9
SD (n = 15)		0.77		0.52
CV, % (n = 15)		0.78		0.54

contents were finely powdered and mixed, and an accurately weighed portion of powder equivalent to 200 mg of I was transferred to a 50-mL volumetric flask. Thirty milliliters of water was added, the mixture was shaken mechanically for 10 min, and then diluted to volume with water. The mixture was filtered (the first 10 mL was discarded).

Injection Assay Preparation—An accurately measured volume of injection solution, equivalent to 200 mg of I, was transferred to a 50-mL volumetric flask, then diluted to volume with water.

Procedure—Assay preparation (2 mL) was tranferred to a 100-mL beaker, 200 μL of hydrochloric acid was added, and the mixture was swirled gently. Three grams of chromatographic diatomaceous earth was added, mixed well with a flexible spatula, and transferred to the column. The beaker was scrubbed with 1 g of diatomaceous earth, which was added to the column, and tamped with a pledget of glass wool. Fifty milliliters of water-saturated ether then four 50-mL portions of water-saturated methylene chloride were passed through the column. The methylene chloride eluants were combined; a 25 mL portion was diluted to 50 mL with methylene chloride.

The absorbance of this solution and the standard preparation were concomitantly determined in 1-cm cells at the wavelength of maximum absorbance, 261 nm, with a spectrophotometer, using methylene chloride as the blank. The quantity (in mg) of I in the portion of the capsules was calculated by $10C(A_u/A_s)$. The quantity (in mg) of I/mL of injection taken was calculated by $(10C/V)(A_u/A_s)$. C is the exact concentration in μg/mL) of I in the standard preparation; V is the volume (in mL) of the injection solution; and A_u and A_s are the absorbances of the sample solution and the standard preparation, respectively.

Assay for 3,4,5-Trimethoxybenzoic Acid (4)—The standard solution was prepared by diluting 25 mg of 3,4,5-trimethoxybenzoic acid to 50 mL with methanol. The bulk drug samples were prepared by transferring 400 mg of I, accurately weighed, to a 10-mL volumetric flask. Methanol was added to volume, and the contents were mixed until a solution was obtained.

Capsule Sample Preparation—An accurately weighed portion of the capsule contents equivalent to 400 mg of I was transferred to a 10-mL volumetric flask. Methanol (~7 mL) was added, and the flask was shaken for ~5 min. The solution was diluted to volume with methanol, mixed, and centrifuged or let stand until the precipitate settled.

Injection Sample Preparation—An accurately measured volume of injection solution equivalent to 200 mg of I was transferred to a separator containing 5 mL of water and 3 mL of 1 M HCl and extracted with two 20-mL portions of ether. The ether extracts were combined and extracted with 10 mL of water. The aqueous layer was discarded, the ether layer was filtered through cotton premoistened with ether into a small glass-stoppered flask and evaporated under a stream of nitrogen, and the residue was dissolved in 2.0 mL of methanol.

Procedure—A 25.0-μL aliquot of bulk drug or capsule sample preparation, or 10.0 μL of injection sample preparation, and 10.0 μL of standard prepa-

Table III—Results for Commercial I Capsules Using the Column Ion-Pair and USP Assay Procedures

Code	Column Ion Pair[a]		USP XX[b]	
	mg/Capsule	Percent of Label	mg/Capsule	Percent of Label
		100-mg Capsule		
A				
Mean	100.8	100.8	103.5	103.5
SD	0.32	0.32	0.31	0.31
CV, %	0.32	0.32	0.30	0.30
High	101.2	101.2	103.8	103.8
Low	100.1	100.1	103.2	103.2
B				
Mean	100.5	100.5	101.6	101.6
SD	0.35	0.35	0.67	0.67
CV, %	0.34	0.34	0.66	0.66
High	101.3	101.3	102.0	102.0
Low	100.1	100.1	100.8	100.8
C				
Mean	99.8	99.8	101.8	101.8
SD	0.37	0.37	0.59	0.59
CV, %	0.37	0.37	0.58	0.58
High	100.4	100.4	102.2	102.2
Low	99.3	99.3	101.1	101.1
		250-mg Capsule		
D				
Mean	255.8	102.3	260.1	104.0
SD	1.59	0.64	1.73	0.70
CV, %	0.62	0.63	0.67	0.67
High	258.1	103.2	261.1	104.4
Low	253.1	101.2	258.1	103.2
E				
Mean	253.6	101.4	255.2	102.1
SD	1.58	0.61	1.60	0.65
CV, %	0.62	0.60	0.63	0.64
High	255.6	102.2	256.8	102.7
Low	250.9	100.4	253.6	101.4
F				
Mean	250.8	100.3	258.1	103.3
SD	1.79	0.72	3.35	1.35
CV, %	0.71	0.72	1.30	1.39
High	253.2	101.3	261.4	104.6
Low	248.1	99.2	254.7	101.9

[a] n = 10. [b] n = 3.

Table IV—Results for Commercial I Injections Using the Column Ion-Pair, PF, and USP XX Assay Procedures [a]

Container and Code	Column Ion Pair[b]		PF[b]		USP XX[c]	
	mg/2 mL	Percent of Label	mg/2 mL	Percent of Label	mg/2 mL	Percent of Label
Ampule						
A						
Mean	202.8	101.4	200.5	100.2	195.0[d]	97.5
Range	200.7–205.4	100.4–102.7	198.6–201.5	99.3–100.8	192.8–196.8	96.4–98.4
SD	1.69	0.82	0.86	0.44	1.78	0.89
CV, %	0.83	0.81	0.43	0.44	0.91	0.91
B						
Mean	201.0	100.5	200.8	100.4	196.2	98.1
Range	199.9–204.1	100.0–102.0	200.3–201.4	100.2–100.7	195.5–197.3	97.8–98.6
SD	1.46	0.70	0.40	0.19	0.95	0.42
CV, %	0.72	0.69	0.20	0.19	0.48	0.42
C						
Mean	199.3	99.7	198.6	99.3	193.3	96.6
Range	197.6–201.5	98.8–100.8	196.9–200.6	98.4–100.3	192.9–193.6	96.4–96.8
SD	1.60	0.82	1.12	0.58	0.35	0.20
CV, %	0.81	0.83	0.56	0.59	0.18	0.21
Syringe						
A						
Mean	206.1	103.1	201.1	100.5	198.2[d]	99.4
Range	203.1–209.0	101.6–104.5	199.7–203.7	99.8–101.8	196.8–200.1	98.4–100.0
SD	2.00	0.99	1.20	0.61	1.48	0.72
CV, %	0.97	0.96	0.60	0.60	0.74	0.72
B						
Mean	204.3	102.2	202.1	101.1	196.1	98.0
Range	202.8–206.3	101.4–103.2	199.9–203.9	100.0–102.0	195.8–196.2	97.9–98.1
SD	1.10	0.57	1.39	0.71	0.23	0.11
CV, %	0.54	0.56	0.69	0.70	0.12	0.12
C						
Mean	200.9	100.4	203.1	101.6	196.4	98.2
Range	199.1–202.6	99.6–101.3	202.8–203.9	101.4–102.0	195.4–197.3	97.7–98.6
SD	1.37	0.66	0.47	0.26	0.95	0.45
CV, %	0.68	0.66	0.23	0.25	0.49	0.46
Vial						
A						
Mean	201.5	100.8	200.5	100.2	195.0[d]	97.5
Range	198.7–203.0	99.4–101.5	194.9–202.3	97.4–101.2	191.8–196.4	95.9–98.2
SD	1.51	0.73	2.12	1.09	2.15	1.09
CV, %	0.75	0.73	1.06	1.09	1.10	1.12
B						
Mean	199.4	99.7	201.4	100.7	197.2	98.6
Range	197.2–200.3	98.6–100.2	199.4–203.1	99.7–101.6	195.7–197.9	97.8–99.0
SD	0.87	0.45	0.94	0.49	1.27	0.69
CV, %	0.44	0.46	0.47	0.48	0.64	0.70
C						
Mean	200.9	100.5	196.4	98.2	192.7	96.3
Range	198.3–202.7	99.2–101.4	194.4–198.1	97.2–99.0	190.4–194.0	95.2–97.0
SD	1.44	0.72	1.08	0.52	2.00	0.99
CV, %	0.72	0.72	0.55	0.53	1.04	1.02

[a] Labeled to contain 200 mg of trimethobenzamide HCl/2 mL. [b] $n = 10$. [c] $n = 3$. [d] $n = 4$.

ration were applied ~2.5 cm from the bottom of a TLC plate coated with a 250-μm layer of fluorescent silica gel. The plate was developed in a mixture of pentane–acetic acid (88:12) in a suitable unlined chamber until the solvent had ascended 10 cm above the spotting line. The plate was removed, dried in air, and observed under short-wavelength UV light. The R_f value of II is ~0.3; I remains on the spotting line.

RESULTS AND DISCUSSION

Assay Method—The compositing method herein includes a "finely powder" step not usually included for capsules. This was necessary because the varying particle sizes of the components caused stratification of the composite, resulting in nonuniform sample portions.

Methylene chloride was chosen as the eluting solvent rather than chloroform, even though the solubility of I is over fourfold greater in chloroform than in methylene chloride[6]. Because the UV cutoff of $CHCl_3$ is at ~245 nm, it is a poor solvent for the UV determination of I, whose maximum and minimum are at 261 and 240 nm, respectively. If chloroform were used as an eluting solvent and then evaporated so that the residue could be dissolved in methanol or 0.1 M HCl, breakdown could occur during heating and evaporating, catalyzed by traces of HCl eluting from the column with the $CHCl_3$. The UV cutoff of CH_2Cl_2 is ~230 nm, and by using it as the eluant, the evaporation and redissolution steps are eliminated, as is the opportunity for decomposition. The FDA Compendial Monograph Evaluation and Development (CMED) program guidelines require that at least 95% of the drug be eluted in the first half of the eluate when column chromatography is used. Analysis of fractions of eluate of bulk I taken through the column ion-pair elution show that methylene chloride is a satisfactory solvent for meeting this requirement.

Statistical results comparing replicate recoveries of bulk I using the column ion-pair, PF, and the USP injection assay procedures are shown in Table I. Recoveries average 99.4% for the column ion-pair procedure, which is ~2–3% higher than recoveries by the PF and USP methods. Linearity of the column ion-pair and PF techniques are shown in Table II. Both methods were found to be equally linear throughout the ranges tested, *i.e.*, 4–12 mg for the column ion-pair and 122–284 mg for the PF methods. Assay results for commercial capsules are shown in Table III, with statistical comparison of the proposed and USP methods. Results are higher using the USP method, but since this is a direct dilution with no cleanup other than filtration, it was expected. Table IV shows results of injection preparation assays by the ion-pair, USP, and PF methods. Both the column ion-pair and PF results are higher than those for the USP method for all lots tested, indicating improved recoveries.

Collaborative Study—All samples for the study were distributed as coded, blind duplicates. Capsule samples were sent to the collaborators as powders. Capsule sample A was prepared by finely powdering and blending 240 commercial I capsules[7] labeled to contain 250 mg of I/capsule. Capsule sample B was composited by finely powdering and blending the contents of 390 capsules[7] of I labeled to contain 100 mg of I/capsule. Capsule sample C was an "authentic" mixture prepared by finely powdering and blending I with the

[6] Unpublished results.

[7] Beecham–Massengill, Inc., Bristol, Tenn.

Table V—Collaborative Capsule Assay Results Using the Ion-Pair Method

Lab.	Composite A, % of Label[b]		Composite B, % of Label[c]		Composite C, % Theoretical[d]	
1	101.8	101.4	100.6	100.5	98.1	98.5
2	98.3	98.4	97.5	94.5	96.7	98.3
3	100.5	101.6	120.2[a]	99.9[a]	100.0[a]	99.9[a]
4	98.0	99.4	98.8	98.7	97.9	98.1
5	99.9	103.0	100.7	100.1	97.8	97.1
6	102.5	102.0	101.3	98.0	98.0	96.9
Mean	100.5		99.1		97.7	
SD	1.75		2.05		0.62	
CV	1.74		2.07		0.63	

[a] Outliers by Dixon test; not included in mean, *SD*, or *CV* calculations (5). [b] Commercial 250-mg capsule. [c] Commercial 100-mg capsule. [d] Authentic sample, 480 mg of I/g.

Table VI—Collaborative Injection Assay Results Using the Ion-Pair Method [a]

Lab.	Ampule, % of Theoretical[b]		Vial, % of Label[c]		Syringe, % of Label[d]	
1	98.9	99.3	99.8	101.2	102.7	102.9
2	96.9	97.1	97.7	97.3	100.1	98.9
3	97.6	98.6	97.7	99.4	102.2	101.6
4	101.0	101.3	102.6	103.1	104.1	105.1
5	99.0	98.6	99.3	99.7	101.9	103.1
6	98.7	100.6	100.8	100.8	103.9	102.5
Mean	99.0		100.0		102.4	
SD	1.42		1.86		1.70	
CV	1.43		1.86		1.66	

[a] Labeled as containing 200 mg of I/2 mL. [b] Authentic sample, composite A. [c] Commercial sample, composite B. [d] Commercial sample, composite C.

manufacturer's designated excipients to achieve a concentration of ~450 mg of I/g and an amount of II equal to 0.25% of the weight of I. Injection solution sample A was an authentic mixture simulating the ampule formulation of I plus II equal to 0.25% of the weight of I. Injection solution samples B and C were composites of 22 20-mL vials[7] and 168 2-mL disposable syringes[7], respectively, each labeled to contain 100 mg of I/mL. Bulk drug samples contained I with 0.8% of added II, I with 0.25% of II, or I alone.

Collaborative assay results are presented in Tables V and VI. Average results for both capsules and injection solutions ranged from 97.7 to 102.4% (recovered or of theoretical) with coefficients of variation of ≤2.07%, indicating good recovery and reproducibility.

None of the commercial lots of the bulk drug, capsules, or injection preparations of I were found to contain II, but the TLC impurity test was developed and included in the collaborative study because it is a rapid and sensitive method for detecting degradation of I. Collaborators reported results on a basis of ≥0.5% of the amount of I, <0.5% of the amount of I, and no II. One collaborator reported incorrectly that one of the 0.8% bulk drug samples was <0.5% and another analyst incorrectly reported that both authentic injection samples had ≥0.5%. Otherwise, the results correlated well.

CONCLUSIONS

The back-extraction method was studied by the author prior to its publication in PF and was found to be a definite improvement over the USP injection assay method. However, a method was desired that would be specific and stability indicating for I, be applicable to assays of capsule as well as injection formulations, and eliminate the problems inherent in separator extractions with ether. The ion-pair chromatography method has been demonstrated by both statistical and collaborative studies to have achieved these purposes. In addition, the proposed TLC procedure has been shown to be a rapid, sensitive, and reliable method for detecting the presence of II, an impurity of I, in bulk drug and formulations.

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