Assay of Official Injections of Amine Salts

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Abstract □ A modification of a method previously suggested for mephentermine sulfate injection was applied to some official injections containing at least 20 mg./ml. of amine salt, the amine base of which is soluble and stable in chloroform. The method involves: (*a*) distributing the aqueous sample over a mixture of chromatographic magnesium oxide and high flow rate, purified, siliceous earth held in a coarse-porosity, sintered-glass filtering funnel; (*b*) eluting the liberated base with several small portions of warm chloroform into glacial acetic acid; and (*c*) titrating the eluates with standard 0.1 *N* perchloric acid using *p*-naphtholbenzein indicator.

Keyphrases □ Amine salt injections—titrimetric assay with *p*-naphtholbenzein, compared to potentiometric method □ *p*-Naphtholbenzein—indicator in assay of amine salt injections □ Titrimetry—assay of amine salt official injections with *p*-naphtholbenzein, compared to potentiometric method □ Injections, amine salt—titrimetric assay

A method of assay for injections of amine salts based on a method of tablet assay proposed by Bican-Fister and Andrec (1) was proposed for mephentermine sulfate injection (2) and adopted by USP XVIII. Based on this work, an attempt was made to apply a generalized modification of the method uniformly to some official injections where the amine base was known to be readily extractable into chloroform and stable in chloroform.

Table I—Assay of Bulk Drug Substances

Amine Salt	Assay		Recovery, Indicator
	Potentiometric	Indicator	
Codeine phosphate	99.15 ± 0.04	99.26 ± 0.04	100.1 ± 2.6[a]
Diphenhydramine hydrochloride	99.6 ± 0.8	100.3 ± 0.5	99.2 ± 0.2
Methoxamine hydrochloride	99.1 ± 0.6	99.2 ± 0.3	99.2 ± 1.1
Quinidine gluconate	100.03 ± 0.06	100.03 ± 0.06	100.1 ± 0.4
Trimethobenzamide hydrochloride	99.4 ± 0.3	99.25 ± 0.08	99.4 ± 0.6

[a] Value obtained from 12 assays by three different analysts.

Table II—Assay Results from Commercial Injectables

Product Composition	Recovery, Percent of Claim	
	Potentiometric	Indicator
Codeine phosphate, 60 mg./ml. 0.5% chlorobutanol, 0.2% sodium bisulfite	96.8 ± 0.7	96.7 ± 0.3
Codeine phosphate, 60 mg./ml. 0.1% sodium sulfoxylate, 0.1% sodium bisulfite, 0.5% phenol, citrate buffer	104.5 ± 0.1	104.9 ± 0.3
Codeine phosphate, 30 mg./ml. 0.5% chlorobutanol, 0.1% sodium bisulfite	97.42 ± 0.04	97.82 ± 0.00
Diphenhydramine hydrochloride injection, 50 mg./ml.[a]	98.5 ± 0.8	99.0 ± 0.9
Methoxamine hydrochloride injection, 20 mg./ml.[b] 0.1% potassium metabisulfite, 0.3% citric acid, 0.3% sodium citrate	98.6 ± 0.5	98.3 ± 0.3
Quinidine gluconate injection, 80 mg./ml.[c] 1% monothioglycerol, 0.25% phenol	100.2 ± 0.3	100.2 ± 0.3
Trimethobenzamide hydrochloride injection NF[d] 0.45% phenol, 0.5 mg./ml. sodium citrate, 0.2 mg./ml. citric acid	102.1 ± 0.3	102.2 ± 0.0

[a] Benadryl, Parke-Davis. [b] Vasoxyl, Burroughs Wellcome. [c] Eli Lilly. [d] Tigan, Roche.

EXPERIMENTAL[1]

Reagents—The following were used: (*a*) 0.1 *N* perchloric acid (mix 8.5 ml. of 70–72% perchloric acid with enough glacial acetic acid to make 1 l., and standardize against primary standard potassium hydrogen phthalate); (*b*) magnesium oxide prepared for chromatography; (*c*) purified siliceous earth[2] with the highest possible flow rate; and (*d*) *p*-naphtholbenzein T.S., USP XVIII, 0.25% in glacial acetic acid.

Procedure—To a coarse-frit, sintered-glass filtering funnel of about 60-ml. volume, add 1 g. of a mixture of 100 g. of purified siliceous earth and 10 g. of magnesium oxide, plus 1 g. of the mixture for each milliliter of injection taken as the sample. Distribute the sample containing about 0.6 meq. of amine over the solid by mixing with a glass rod. Extract the material in the crucible with five 10-ml. portions of warm (55°) chloroform by mixing each portion thoroughly with the crucible contents and then draining with the aid of gentle suction. Collect the eluates in a 250-ml. suction flask containing 40 ml. of glacial acetic acid. Add 6 drops of *p*-naphthol-

[1] An Orion research digital pH meter, model 801, with an Ingold combination glass electrode (silver–silver chloride) was used.

[2] Celite 545, Johns-Manville Co.

benzein indicator and titrate with standard 0.1 *N* perchloric acid to a green end-point. Perform a blank determination and make any necessary corrections.

Recoveries were determined by first determining the purity of the crystalline salt by titration in triplicate with 0.1 *N* acetous perchloric acid, adding mercurous acetate prior to titration for the hydrochloride salts, and using *p*-naphtholbenzein indicator simultaneously with potentiometric determination of the end-point. An aqueous solution of the amine salt was then prepared at the same concentration as a commercial product and assayed in triplicate by the described method, and the recovery was determined. A commercial product was then assayed by the method; recovery in terms of percent of label claim was determined, both colorimetrically and simultaneously potentiometrically to show the effect of possible interferences with the indicator.

RESULTS

Results are summarized in Tables I and II.

DISCUSSION

Agreement between assays using potentiometric and indicator end-points is good, and all recoveries from a simple aqueous solution of the salt are good. The close agreement between potentiometric and indicator end-points for the pure compounds shows that *p*-naphtholbenzein is a proper indicator for the titration.

The results show the general utility of the method for assay of simple aqueous solutions and compounded injections of amine salts that meet the following criteria: (*a*) concentration of 20 mg./ml. or more, (*b*) amine base readily extractable into chloroform, and (*c*) amine base stable in chloroform.

Preliminary experiments showed that a large aqueous sample necessitated by low concentration led to erratic recoveries and that attempts with smaller samples using 0.01 *N* perchloric acid as the titrant also failed to yield reliably reproducible results.

Attention is called to the rapidity of the method, the low volume of extracting solvent used, and the complete freedom from emulsion formation as compared with methods involving solvent extraction.

REFERENCES

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TECHNICAL ARTICLES

Automated Turbidimetric Microbiological Assay Readout System▲

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Abstract □ A new automated turbidimetric assay readout system utilizing commercially available off-the-shelf equipment is described. This system is capable of obtaining the absorbance values of over 400 individual tubes virtually unattended and is being used routinely in the certification of antibiotics with greater precision and speed than was previously attainable. Because the system involves no change in basic methodology from the current official procedure for antibiotic assay, no change in the "Code of Federal Regulations" is required.

Keyphrases □ Automated analysis—turbidimetric microbiological assay □ Microbiological assay—automated turbidimetric readout system □ Turbidimetric microbiological assay—automated readout system □ Antibiotics—automated bioassay readout

During the last several years the pharmaceutical industry has expended large amounts of time, energy, and resources in efforts to automate many of the more repetitive, time-consuming, and tedious aspects of microbiological assays of antibiotics and vitamins. Kuzel and Roudebush (1) described their experiences with several combinations of spectrophotometers and flow cells. Kuzel and Coffey (2) described a unique cell positioner which permitted two flow cells to be monitored by one spectrophotometer without large variations of blank readings due to changes of cell position in the spectrophotometer. Other investigators (3) used three matched flow cells to determine the transmittance readings of three-dose turbidimetric assays of vitamins, with one dose level always being read from the same flow cell to minimize carryover. This system employed a digital converter and a computer card punch on the output end.

Burns and Hansen (4) devised a system which accepted whole fermentation beers, filtered and diluted them, mixed the diluted sample with medium and inoculum, and dispensed it into a continuous chain of 2-ml. cups inside a 37° incubator. The chain was long enough so that when the desired period of incubation was com-