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Colorimetric determination of amphetamine salts in dosage forms

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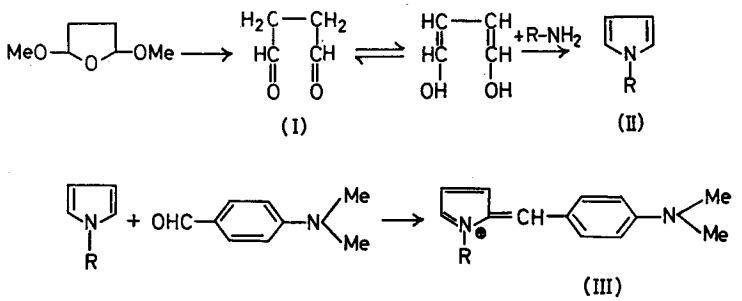
A colorimetric method for the determination of primary amines has been applied to the assay of amphetamine salts in dosage forms. Aqueous solutions of amphetamine salts heated in acidic medium with 2,5-dimethoxytetrahydrofuran and subsequently reacted with *p*-(dimethylamino)benzaldehyde, give a red colour. This reaction is useful for photometric determinations at 557 nm. Secondary and tertiary amines do not interfere.

Amphetamine salts in dosage forms may be determined by spectrophotometric methods, by chromatographic procedures or by steam distillation from alkaline medium and titrimetric assay of the distillate. In this last method (British Pharmacopoeia, 1968) a large sample is required and volatile bases interfere. Rarely are the spectrophotometric methods (Ebstein & Van Meter, 1952; Milos, 1960) useful in the assay of amphetamine dosage forms, because the molar extinction of the drug is low and many other drugs associated with amphetamine, cause interference.

Although chromatographic methods (Beckett & Rowland, 1965; Cardini, Quercia & Calò, 1967; Choulis & Carey, 1968) can be used successfully in analyses of pharmaceutical preparations, a simple and fast colorimetric method would also be very useful for routine amphetamine assay in dosage forms. Our attempts to use the procedures proposed by Wachsmuth & Van Koeckhoven (1964; 1965) were unsuccessful since the sensitivity and the reproducibility of the methods were not good enough. Sawicki & Johnson (1966) reported a colorimetric method for the determination of primary amines. In this procedure $R–NH_2$ reacts with succinaldehyde (I) generated *in situ* from 2,5-dimethoxytetrahydrofuran to give the *N*-substituted pyrrole (II). The reaction between this compound and *p*-(dimethylamino)benzaldehyde produces the chromogen (III).



We have applied this method to amphetamine assay in dosage forms and investigated the variables in the determination.

EXPERIMENTAL

Reagents. 0·5% solution of 2,5-dimethoxytetrahydrofuran (Aldrich-Chemical Co.) in glacial acetic acid. 2·0% solution of *p*-(dimethylamino)benzaldehyde (Merck) in glacial acetic acid–concentrated hydrochloric acid (85:15 v/v). 0·025 mg/ml standard amphetamine sulphate aqueous solution.

A "Densitronic" (Optica, Milan) spectrophotometer with sample path length of 10 mm was used.

Sample preparation. All the samples are diluted so that the final solutions contain about 0·025 mg/ml of amphetamine salt.

Tablets (amphetamine sulphate = 5·0 mg): powder 20 tablets, add water to a weighed amount of powder and dilute to volume with water. Mix and filter.

Injections (amphetamine sulphate = 10·0 mg/ml): dilute a measured volume of sample to volume with water.

Suppositories (amphetamine sulphate = 5·0 mg + dimenhydrinate 100 mg): cut 10 suppositories into small pieces and dissolve a weighed amount in chloroform, extract with three equal quantities of water, combine the aqueous extracts, dilute to volume with water, mix and filter.

Assay

Into three glass stoppered tubes (20 × 100 mm) introduce respectively 2 ml of sample solution, 2 ml of standard solution and 2 ml of water. Add 1 ml of 2,5-dimethoxytethrahydrofuran solution to each tube. Place the tubes simultaneously in a boiling water bath and, 30 s later, stopper the tubes. Heat for 20 min. Remove the tubes from the bath and cool them in iced water. Add 2 ml of *p*-(dimethylamino)-benzaldehyde solution to each tube, stopper again and heat in boiling water bath for 2 min. Gently shake the tubes, place them in cool water and, 10 min later, read the extinctions of standard and sample solutions at 557 nm against the blank.

RESULTS

The extinction spectrum of chromogen solution shows maxima at 517 and at 557 nm. A linear relation exists at 557 nm between extinction value and drug concentration but the values are not exactly reproducible and an assay on a standard solution must be run alongside each sample.

The recoveries obtained in ten repeated analyses of a standard solution of amphetamine sulphate containing 0·025 mg/ml, gave a mean value of 0·0256 with a relative standard deviation of 0·0007 (2·73%). The results obtained in comparative analyses of different lots of dosage forms (Table 1) show that the proposed colorimetric procedure yields results which are in agreement with those obtained using volumetric or gas chromatographic methods.

The optimum conditions for the method were sought with the following findings. (i) The specific absorption values at 557 nm were more constant than those at 517 nm. (ii) Optimum results were obtained with a 0·5% solution of 2,5-dimethoxytetrahydrofuran. Higher concentrations gave lower results. (iii) By heating the sample in boiling water for 20–30 min the transformation of amphetamine into *N*-substituted pyrrole took place with maximum yield (10 min of heating gave 3/4 of maximum yield). (iv) Maximum colour development took place when 2% solution of *p*-(dimethylamino)benzaldehyde in 85:15 mixture of glacial acetic acid and concentrated

Table 1. *Comparative assays of amphetamine sulphate carried out in duplicate with the proposed procedure (A) and with published procedures (B, C, D) on homogenized samples obtained from different batches of dosage forms.*

Samples	5 mg Tablets A mg/t	5 mg Tablets B mg/t	10 mg/ml Injections A mg/ml	10 mg/ml Injections C mg/ml	5 mg Suppositories (+ 100 mg dimenhydrinate) A mg/s	5 mg Suppositories (+ 100 mg dimenhydrinate) D mg/s
Lot I	4·70	4·88	10·03	9·80	5·00	4·98
Lot II	5·10	4·90	9·86	10·30	5·03	4·86
Lot III	4·90	5·10	10·03	9·81	4·96	5·04
Lot IV	4·87	4·74	10·08	9·64	4·90	4·95
Lot V	4·97	4·80	9·86	10·30	5·19	4·98
$\bar{X}$	4·908	4·884	9·972	9·970	5·016	4·962
s.e.	0·065	0·061	0·046	0·138	0·048	0·029
Average of differences*	—0·024 ± 0·088		—0·002 ± 0·184		—0·054 ± 0·058	

A = Proposed colorimetric procedure. B = Volumetric B.P. 1968 (p. 45) method.
C = Volumetric U.S.P. XVI (p. 55) method. D = Gas chromatographic procedure proposed by Cardini, Quercia, & Calò (1967).

* = The standard error refers to the average of differences obtained in each pair.

hydrochloric acid was used. An increased percentage of acetic acid in the mixture (i.e. 95:5) gave negligible variation, but if the amount of hydrochloric acid was increased, results were lower (60:40 mixture gave half maximum colour). (v) By heating the sample from 1 to 3 min in boiling water after the *p*-(dimethylamino)-benzaldehyde was added, the maximum colour was obtained. Longer heating times gave low colours (80% after 5 min and 60% after 10 min). (vi) The colour stability is good between the 5th and the 12th min after heating. In this period the highest extinction is read. Afterwards the colour fades slowly at a rate of about 6% of absorbance per 10 min during the first hour. The colour of the sample and of the standard fade at the same rate, preventing biased results. The colour is more stable if the solutions are put on ice rather than in cool water. Storage in the dark did not affect the fading.

The following compounds, sometimes associated with amphetamine in dosage forms, gave no interferences in the assay: dimenhydrinate, diphenhydramine hydrochloride, phenylephrine hydrochloride, methamphetamine hydrochloride, adrenaline bitartrate, diphenylhydantoin, mephobarbital.

Imidazole and purine derivatives and secondary and tertiary amines do not undergo the reaction and do not interfere. Primary aliphatic and aromatic amines, including amino-acids, do interfere.

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