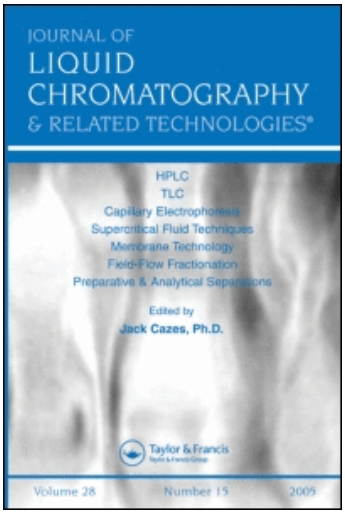

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HPLC QUANTIFICATION OF AZINTAMIDE AND PAPAVERINE HYDROCHLORIDE SIMULTANEOUSLY IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

A simple HPLC-procedure for quantification of azintamide and papaverine.HCl simultaneouly in dosage formulations has been investigated. The complete resolution and quantitative determination of both drug substances has been undertaken on a Hibar 100RP-18 Lichrospher (5 µm) column by using a solvent mixture composed of acetonirile - water (56:44, v/v) isocratically at a rate of 1 $ml.min^{-1}$ with UV-detection at 240 nm. Recovery percentages of 100.39 ± 0.70 (n = 12) and 99.97 ± 1.11 (n = 12) were obtained for added azintamide and papaverine.HCl, in order.

INTRODUCTION

Azintamide, 2-((6-chloro-3-pyridazinyl)thio)-N,N-diethylacetamide, is a synthetic potent choleretic drug substance with moderate cholepoetic action. The drug is commonly prescribed for the treatment of fatty indigestion, as antihypercholesteremic, and in some other cases such as cholecystitis, cholangitis, icteric and posticteric conditions. Papaverine hydrochloride is commonly dispensed together with azintamide,

for its smooth muscle relaxation, to inhibit possible spasmomimetic activity of azintamide in the gastrointestinal tract.

Various analytical techniques, including colorimetry (1), UV-spectrophotometry (1,2), PMR-spectrophotometry (3), gas-liquid chromatography (GC) (4), high-performance liquid chromatography (HPLC) (5), and flow-injection analysis (FIA) with UV-detection (2) have been described for determination of azintamide in bulk materials and in some of its single-component dosage formulations.

Sensitive methods for determination of the choleretic drug azintamide in admixtures with the spasmolytic alkaloid papaverine hydrochloride are always required. High-performance liquid chromatography promises to be one of the most effective techniques ever devised for separation of complex mixtures in being sensitive and versatile. The present communication recommends an evaluated HPLC-procedure for accurate determination of both drug substances contemporaneously in dosage forms. The results obtained have been statistically compared with those of another derivative-spectrophotometric procedure (7).

EXPERIMENTAL

Apparatus

A stainless steel, 12.5 cm X 4 mm i.d.; LC-column packed with Hibar 100RP-18 Lichrospher (5 µm), E. Merck, Darmstadt-F.R.G., installed in a Varian model 5000 Liquid Chromatograph equipped with a variable Varian UV-50 detector set at 240 nm, a Rheodyne model 7125 injector with a 20-µl sample loop, and a Varian model 9176 strip recorder (chart speed of 1 $cm.min^{-1}$), were used. The chromatographic parameters were controlled by a Varian Data System model DCS 111L. The flow rate of the

eluting solvent mixture, acetonitrile - water, (56:44, v/v) was 1 $ml.min^{-1}$.

Reference materials and chemicals

Azintamide (98.91% pure as assessed by GLC-analysis) (4) under the trade name Oragallin from Chemie Linz AG, Linz/Donau-A., B. No. 23540/524699 and papaverine hydrochloride (99.72% labelled purity), Carlo-Erba, Milano-I., B. No. 382218-24676, were used as reference substances without further treatment. HiPer Solv acetonitrile for HPLC, BDH-Chemicals, Poole-England, and all-glass distilled water were used for preparing the mobile phase.

Pharmaceutical preparations

Oragallin comp.® dragées, manufactured by Chemie Linz AG, Linz/Donau-A, each sugar-coated dragée claiming to contain 100 mg azintamide and 10 mg papaverine hydrochloride, B.No.: 232631, were purchased from local pharmacies in Riyadh - K.S.A.

Standard solutions and curves

The stock solutions of azintamide, 156 $\mu g.ml^{-1}$ and papaverine.HCl, 152 $\mu g.ml^{-1}$ in the mobile phase, were diluted to 39 $\mu g.ml^{-1}$ and 7.6 $\mu g.ml^{-1}$, respectively, as working solutions. To prepare the standard curves, serial dilutions containing azintamide, 1.56-7.80 $\mu g.ml^{-1}$, and papaverine.HCl, 0.30-1.52 $\mu g.ml^{-1}$, were obtained by diluting the working solutions. Triplicate injections of each standard solution were made and curves for each drug substance, concentration *vs* detector response, were plotted. The slope consistency of the prepared curves for each drug was checked at different days.

Analysis of Tablets

Twenty tablets were accurately weighed and powdered. An aliquot of the powder claimed to contain 10 mg azintamide and consequently 1 mg

of papaverine hydrochloride was transferred into a 100-ml calibrated flask. About 80 ml of the mobile phase were then added and extraction was allowed by mechanical shaking for about 15 minutes. The volume was completed and the solution was filtered. 1-ml Portions were diluted to 20 ml (to give final concentrations of 5 $\mu g.ml^{-1}$ azintamide and 0.5 $\mu g.ml^{-1}$ papaverine HCl), then 6 injections of each solution were made onto the LC-column.

To determine the component drug ingredients in dosage formulations, refer either to prepared calibration curves or/and direct sample to equivalent standard comparison.

RESULTS AND DISCUSSION

The liquid chromatographic conditions recommended for quantification of azintamide in single-component pharmaceutical formulations on a Novapack-C18 (5 µm) using a mixture of methanol and water, 98:2, v/v, isocratically (5) was tried. The method was found unsuitable for separation of papaverine hydrochloride in admixtures with azintamide.

Trials have been undertaken in order of achieving the best LC-conditions for separation and quantification of azintamide and papaverine hydrochloride in laboratory prepared binary mixtures. The preliminary investigations showed the suitability of the reversed-phase 100 RP-18 LiChrosphere (5 µm) column for such aim.

Elutions using different proportions of methanol and 0.05% ortho-phosphoric acid achieved excellent separation of only azintamide from binary mixtures. Increasing the amount of the acid in the mobile phase showed equivalent decreases in the retention of azintamide; but the other drug component, i.e. papaverine salt, still unresolved. A

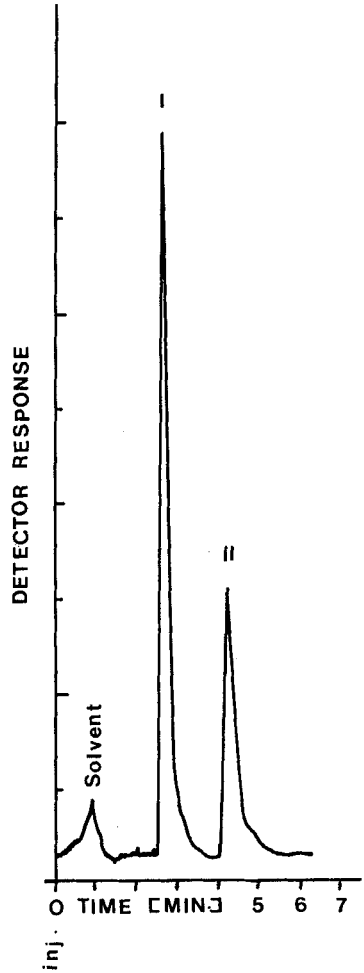

Figure. 1: Typical HPLC-resolution of azintamide I (5 µg.ml-1 and papaverine.HCl, II (0.5 µg.ml-1) in tablet's extract.

mobile phase containing acetonitrile and water, 56:44, v/v, at flow rate of 1 $ml.min^{-1}$ showed the best separation of both the choleretic and the spasmolytic drugs with retentions of 3 and 5 minutes for azintamide and papaverine hydrochloride, in order. However, increasing the acetonitrile contents in the mobile phase tends to reduce the resolution of the two drugs. The calculated capacity factors for azintamide and papaverine hydrochloride were 2.06 and 3.78, respectively, while the measured resolution, R_s, was 2.96.

UV-scannings of each drug component demonstrate typical light absorption maxima for azintamide at 258 nm [A(1%, 1 cm) = 547] (1) and papaverine hydrochloride at 240 nm [A(1%, 1 cm) = 2342] (6). For UV-detection in HPLC-separation and quantification, the wavelength of 240 nm was found suitable for tracing both components simultaneously because of the unbalanced composition-ratios of azintamide and papaverine hydrochloride, 10:1, in the analyzed dosage formulations. The chosen wavelength allows good detection of both drug components in the same dilution for tablet extracts without affecting the sensitivity of detection of any of the components.

Table 1 demonstrates collectively the obtained HPLC-data from resolved authentic azintamide and papaverine hydrochloride following the described HPLC-procedure. Table 2 shows the assay results for the two drugs in Oragallin comp. ® tablets as well as the recovery testing of different added amounts of azintamide and papaverine hydrochloride to tablet extracts containing both drug substances. The results obtained by adopting the LC-method have been statistically compared with those obtained by applying the first-derivative, $D_1(dA/d\lambda)$ spectrophotometric measurements of azintamide directly at 264 nm and using a worked out simultaneous equation at 244 nm and 254 nm for calculation of papaverine

TABLE 1

HPLC-data for calibrated standard curves of azintamide and papaverine hydrochloride by UV-detection at 240 nm

Drug substance	Concentration (µg.ml-1)	Detection limit (µg.ml-1)	Detection limit (ng-injected)	Regression equation Y = A + BC* A	Regression equation Y = A + BC* B+	r-value
Azintamide	1.50 - 8.00	0.30	15	- 233.6	138707	0.9999
Papaverine.HCl	0.30 - 1.50	0.10	5	- 8429	841864	0.9999

* C = concentration, µg.ml-1
\+ B = average area-count of 1 µg.ml-1 (20 µL = 50 ng of the drug injected).

TABLE 2

Assay and recovery of azintamide and papaverine hydrochloride in/or added to Oragallin comp.® tablets

	Azintamide		Papaverine Hydrochloride	
	HPLC-method	D_1-method[7]	HPLC-method	D_1-method[7]
Assay; X- ± SD	101.03 ± 0.96	100.30 ± 0.94	100.37 ± 0.70	99.99 ± 0.73
n	6	7	5	7
CV	0.95	0.94	0.69	0.73
Student's-t+	1.388 (2.447)		0.918 (2.571)	
Recovery; X- ± SD	100.39 ± 0.70*	101.02 ± 1.61	99.97 ± 1.11*	100.48 ± 1.33
n	12	5	12	5
CV	0.70	1.60	1.11	1.32
Student's-t+	1.134 (2.179)		1.131 (2.179)	

+ Figures in parentheses are the corresponding tabulated-t values at P = 0.05 and n-1 df.

* Amounts of azintamide and papaverine hydrochloride added were 0.78-1.56 $\mu g.ml^{-1}$ and 0.15 -0.30 $\mu g.ml^{-1}$ respectively.

hydrochloride contents (7). The results of both procedures are statistically valid but the LC-method seems to be rapid, simple and more stability-indicating than the spectrophotometric procedure.

The reproducibility of the investigated LC-method was assessed by injecting 10 replicates of a standard mixture containing azintamide (5.5 $\mu g.ml^{-1}$) and papaverine hydrochloride (0.55 $\mu g.ml^{-1}$) i.e. in their same composition ratio in the analyzed tablets. The results showed acceptable coefficiednt of variations of 0.66% and 0.92% for azintamide and papaverine hydrochloride, in order. The reproducibility and accuracy were evaluated also by the obtained consistancy of the slopes of different standard curves prepared at different times. Injected amounts of 15 ng of azintamide and 5 ng of papaverine hydrochloride could be traced by the recorder but with poor accuracy.

As a result of the present work, azintamide and papaverine hydrochloride can be analyzed simultaneously in their binary mixtures and in dosage formulations with high accuracy and reproducibility. The range of drug analysis (few µg.ml-1) may find application for detection and quantification of both drugs in biological fluids. The described LC-method is highly recommended for routine analysis of both drug substances in pharmaceutical drug research and good analytical practice (GAP) in pharmaceutical industry and even for adoption as a standardized pharmacopoeial procedure.

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