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SPECTROFLUORIMETRIC DETERMINATION OF SOME DIBENZAZEPINE DRUGS

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تم دراسة طريقة طيفية لصفية دقيقة وحساسة لستة مركبات من عقاقير ثنائى البنزازيبين ، وهذه المركبات هى هيدروكلوريد كل من الأميبرامين والديسيبرامين والكلوميبرامين ومالييات الترامييرامين وثنائى هيدروكلوريد الأوبيبراموال والكاربامازيبين ، واعتمدت هذه الطريقة على أكسدة المركبات موضع الدراسة باستخدام ثنائى كرومات البوتاسيوم فى وسط حمضى.

وقد تم دراسة الكثافة اللصفية النسبية للمركبات عند طول موجى يتراوح بين ٤٦٠-٤٨٥ ن م. وقد تم تعيين المركبات بنجاح فى تركيزات تتراوح بين ٠,١ - ٠,٣ ميكروجرام لكل ملليليتر.

وتم تطبيق الطريقة فى تعيين تلك المركبات فى المستحضرات الصيدلية فى عينات البلازما ، وكانت النتائج متوافقة مع نتائج الطرق الدستورية.

A convenient and sensitive spectrofluorimetric method for the determination of six dibenzazepine drugs, namely imipramine hydrochloride, desipramine hydrochloride, clomipramine hydrochloride, trimipramine maleate, opipramol dihydrochloride and carbamazepine was investigated. The developed method depends on the oxidation of the compounds with potassium dichromate in acid medium. The relative fluorescence intensities for all the studied drugs were measured at an excitation wavelength range of 350-360 nm and an emission wavelength range of 460-485 nm against a reagent blank prepared similarly. Studied drugs could be determined successfully in the range of 0.1-3.0 μg/ml. The method was satisfactorily applied to the determination of them in pharmaceutical preparations and in spiked plasma samples. The results obtained from proposed method were found in good agreement with those obtained from official methods.

INTRODUCTION

Dibenzazepines are tricyclic anti-depressants, used widely for the treatment of emotional and psychiatric disorders.[1] Carbamazepine is used as anticonvulsant.[2] A variety of methods for determination of these drugs have been developed. These include titrimetry,[2-4] spectrophotometry,[5-13] atomic absorption spectroscopy,[14] fluorimetry,[15-17] polarography,[18-20] flow injection analysis,[21] thin layer chromatography[22,23] and high performance liquid chromatography.[24-29]

In this work we have developed a spectrofluorimetric procedure for determination of the previously mentioned drugs, based on their oxidation with potassium dichromate in dilute sulphuric acid medium. The method possesses distinct advantages over the existing spectrophotometric methods in terms of accuracy, sensitivity and stability of fluorophores. In comparison with the direct native fluorimetric method (emission at 385 nm), the suggested method has the advantage of the measurements at longer wavelengths (460-485 nm) which are far from interference, specially for biological samples. Accordingly, the direct native fluorescence method was not applied for the analysis in biological fluids. The present method has been successfully applied for the accurate determination of the studied drugs both in pharmaceuticals and in spiked plasma without serious interference indicated by the good obtained recoveries.

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EXPERIMENTAL

Apparatus

Spectrofluorometer: SFM 23/B, Kontron, Switzerland and Spectrophotometer: Uvidec-32-, JASCO, Tokyo, Japan.

Chemicals and reagents

Pharmaceutical grade imipramine hydrochloride, desipramine hydrochloride, clomipramine hydrochloride, trimipramine maleate, opipramol dihydrochloride and carbamazepine were obtained as gifts from Ciba-Geigy and Specia. All compounds were complying with the requirements recommended by official methods,[2,3] and used as such without further treatments. All reagents and solvents used throughout this work were analytical grade.
Potassium dichromate solution; 0.02% w/v in distilled water.
Sulphuric acid solution; 0.5 M in distilled water.

Dosage forms

Commercial dosage forms were purchased from the local market and were analyzed by the proposed method.

Plasma samples

Plasma samples were obtained from healthy volunteers advised to avoid smoking, taking drugs or any food preservatives.

Preparation of standard solutions

Into different 50-ml volumetric flask, an accurately weighed 25 mg of each of the studied drugs was dissolved in about 40 ml of water (ethanol in case of carbamazepine), then completed to the volume with the same solvent after complete dissolution. The required working standard solutions were prepared by diluting the proper volume of the stock solution with water to give concentrations in the range of 1-30 μg/ml of each of the studied drugs.

Preparation of the sample solutions

(a) Tablets

Twenty tablets were accurately weighed and finely powdered. An amount of the powdered tablets equivalent to 25 mg of each of the studied drugs was transferred to 50 ml volumetric flask and dissolved in 40 ml of water (ethanol in case of carbamazepine). The mixture was sonicated for few minutes to ensure complete dissolution and then completed to the mark with the same solvent. The solution was filtered and the first portion of the filtrate was discarded. An accurately measured volume of the filtrate was quantitatively diluted with water to obtain the required concentration for determinations.

(b) Biological samples

Spiked plasma samples were prepared by mixing the appropriate volume of standard solutions of trimipramine maleate with drug-free human plasma to produce a concentration range of 1-30 μg/ml of the studied drug. (spiked plasma samples were stored at -20°C until use).

Into a glass centrifuge tube, 4 ml of acetonitrile was added to 1 ml of the spiked plasma sample and vortex-mixed. The precipitated proteins were separated by centrifugation for about 5 minutes (3000 rpm). From the clear acetonitrile layer, the required volume (1 ml) was pippted and used for determinations. For blank experiment 1 ml plasma was similarly treated.

General procedure

An accurately measured 1 ml of either standard or sample solution, 1 ml of sulphuric acid and 0.5 ml of potassium dichromate solution were added sequentially to a 10-ml volumetric flask. The contents of the flask were mixed well and placed in a boiling water bath for about 10 minutes (30 minutes for carbamazepine).

The reaction mixture was cooled and diluted to the mark with water. The relative fluorescence intensity of the solution was measured in a 1-cm quartz cell at an excitation wavelength of 350-360 nm and an emission wavelength of 460-485 nm against a blank solution prepared concurrently.

RESULTS AND DISCUSSION

The present work describes a spectrofluorimetric assay for the six dibenzazepine tricyclic antidepressant drugs in pure form and in some commercial tablets as well as in spicked plasma samples. The method involves the oxidation with potassium dichromate in acid medium to produce fluorescent products. Measurements were conducted at 460-485 nm (excitation at 350-360 nm). In order to investigate that the measured emission is due to the oxidation product, experiments were carried out by recording the emission of desipramine HCl as a representative example of the studied drugs after heating with sulphuric acid, after oxidation with dichromate, and that of the reduction product of dichromate (Cr III). Fig. 1 indicates that the measured fluorescence is that of the oxidation product of desipramine HCl. The general oxidative behaviour of all of the studied compounds was found to be similar.

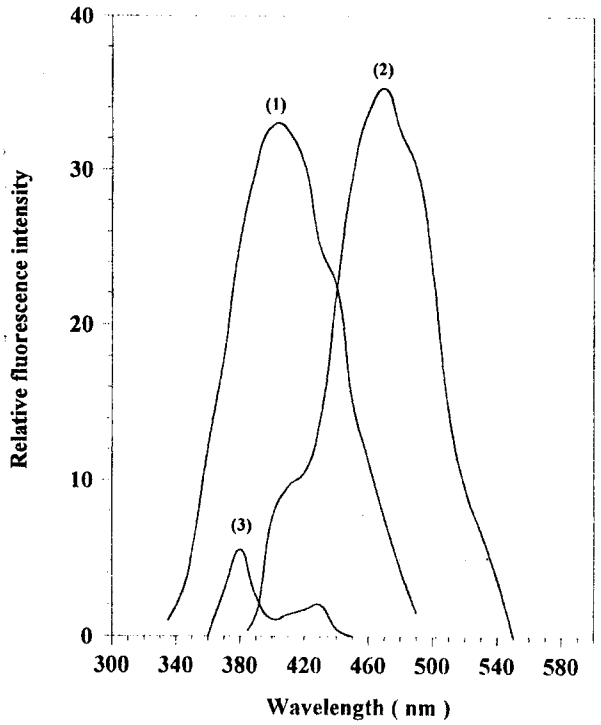

Fig. 1: Emission spectra of (1) desipramine HCl, 2 μg/ml (excitation at 280 nm), (2) its oxidation product (excitation at 350 nm) and (3) chromium (III), 1 μg/ml (excitation at 230 nm).

It was reported that some dibenzazepines could be oxidized using different oxidants; carbamazepine was oxidized with ceric sulphate to a fluorescent product;[15] imipramine, desipramine and trimipramine produced a blue colour when treated with 32% nitric acid[30] and when treated with potassium dichromate in sulphuric acid medium.[9]

In the present work the blue coloured oxidation product of the studied drugs with dichromate is changed to a yellow fluorescent product upon heating. The sequence of the reaction as predicted from literature[9,31] can be explained by the formation of the reactive cation radical followed by fast coupling at position 2 to form unstable intermediate which rapidly gives a blue coloured dimer. Further oxidation of the coloured product yields a more stable yellow coloured fluorescent product, which was measured through this work.

Optimization of variables

Investigations on the influence of different parameters affecting fluorophore development with respect to maximum sensitivity, stability and reproducibility led to the general procedure stated above.

Fig. 2 shows the effect of potassium dichromate concentration on the fluorescence intensity of oxidation product of desipramine HCl as a representative example of the studied drugs. Maximum fluorescence intensity and reproducible results were obtained by using potassium dichromate solution in the range of 0.01-0.05% w/v. Potassium dichromate solution, 0.5 ml of 0.02% w/v, was used throughout the work.

Five acids, namely acetic, hydrochloric, phosphoric, nitric and sulphuric acids were tested in combination with the used oxidant. Sulphuric acid gave the most intense and stable fluorescence. It was found that 1 ml of 0.5 M sulphuric acid gave the maximum fluorescnece intensity.

Water was the best solvent to be used for diluting the reaction mixtures. Other organic solvents such as methanol, ethanol, isopropanol, dimethylformamide, dimethylsulfoxide, 1,4-dioxane and acetonitrile decrease markedly the relative fluorescnece intensity when used instead of water.

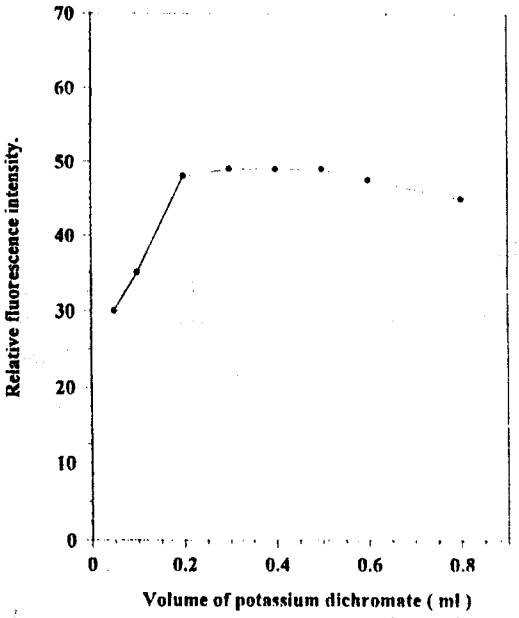

Fig. 2: Effect of volume of potassium dichromate (0.02% w/v) on the relative fluorescence intensity of reaction product of desipramine HCl (2.5 μg/ml) with potassium dichromate.

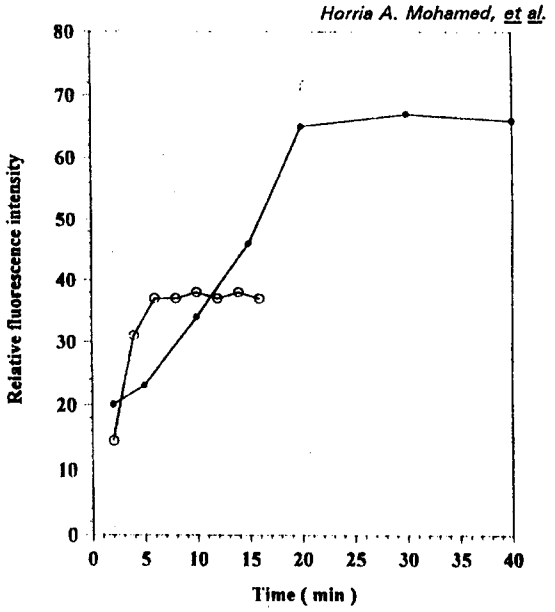

Fig. 3: Effect of heating time (boiling water bath) on the fluorescence of reaction product of carbamazepine (0.3 μg/ml) and desipramine HCl (2 μg/ml) with potassium dichromate (● carbamazepine; ○ desipramine HCl).

The optimal time required to give maximum fluorescence intensity was determined by following the reaction at 25°C and at 100°C (boiling water bath). At 25°C, the reaction is not complete, even after standing for 24 hours. Fig. 3 shows that, heating on a boiling water bath was essential for completeness of the reaction. Fluorescence intensity for all studied drugs except carbamazepine were reached maximum after 5 minutes at 100°C and remained stable for further 30 minutes. In case of carbamazepine fluorescence readings were reached maximum after about 20 minutes and remained stable for further 40 minutes. Ten minutes was selected as the optimal heating time for all studied drugs (30 minutes in case of carbamazepine).

In all cases, after dilution of the reaction mixture with water, the fluorescence intensity remained constant for at least 2 hours.

Under the experimental conditions described above, the relationship between relative fluorescence intensity and the concentration for all studied drugs was linear in the general range of 0.2-3.0 μg/ml of the final measured solutions. Table 1 shows the regression statistics for the studied drugs.

Commercial dosage forms containing the studied drugs were successfully analyzed by the proposed method. The results were compared with those obtained by applying the official methods.[2,3]

In the t- and F-tests, there were no significant differences between the calculated and theoretical values (95% confidence limit) for comparison of the proposed method with the official ones (Table 2), indicating similar accuracy and precision. Recovery experiments were carried out for studied drugs in different preparations. The excellent recoveries (98%) obtained indicate the absence of interference from the commonly encountered pharmaceutical additives.

Spiked plasma samples containing trimipramine maleate in three different concentrations (0.5, 1.5 and 2.5 μg/ml) comparable to those used for preparation of standard solutions were subjected also to analysis under the same conditions used in the proposed method after separation of insoluble proteins.

Table 1: Statistical characteristics for the method of analysis of the studied dibenzazepine drugs.

Drug	Calibration rang μg/ml	Intercept ±SD	Slope ±SD	Correlation coefficient	Detection limit* μg/ml
Imipramine hydrochloride	0.50-3.0	-0.3±0.92	21.17±0.47	0.9990	0.144
Clomipramine hydrochloride	0.25-2.0	0.66±0.51	29.48±0.41	0.9994	0.074
Desipramine hydrochloride	0.5.-2.5	-1.65±0.42	19.70±0.25	0.9998	0.146
Opipramole dihydrochloride	0.20-1.2	1.27±0.82	48.07±1.05	0.9990	0.076
Trimipramine maleate	0.25-2.0	-0.75±0.24	21.67±0.19	0.9998	0.068
Carbanazepine	0.10-0.5	-1.45±1.1	211.5±3.30	0.9996	0.022

*Detection limit was calculated according to reference 32.

Table 2: Application of the method for the determination of some of the studied tricyclic dibenzazepine drugs in tablets.

Sample	Drug content (mg)	%Recovery* ±SD	Official**
Tofranil tablets (Imipramine HCl)	25	97.95±0.40 F= 3.24, t= 1.49	98.5±0.72
Anafranil tablets (Clomipramine HCl)	25	98.31±0.46 F= 1.5, t= 0.712	98.5±0.38
Isidone tablets (Opipramole dihydrochloride)	50	98.29±0.85 F= 1.68, t= 0.186	98.0±1.1***
Tegretol CR tablets (Carbamazepine)	200	98.75±0.31 F= 3.75, t= 0.331	99.0±0.6

* Average of 5 determinations. Theoretical values at 95% confidence limit : F= 6.39 and t= 2.78.
** According to Ref. 2.
*** According to reported methoed Ref. 17.

Excellent recoveries were obtained (98.20±1.2, 98.4±1.1 and 98.86±0.78 for the three concentrations 0.5, 1.5 and 2.5 μg/ml respectively) indicating the absence of interferences from proteins and other components of plasma.

As a conclusion, the proposed method is simple, sensitive and reproducible. It is quite useful for analysis of the studied dibenzazepines in pharmaceuticals as well as blood plasma.

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