RESEARCH PAPER

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Determination of Propyphenazone and Ketoprofen by Quantitative Thin Layer Chromatography and High Performance Thin Layer Chromatography

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A quantitative thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC densitometry) procedures have been developed for the determination of propyphenazone and ketoprofen in formulations. These two constituents were separated by TLC techniques followed by extraction of the components with methanol and the measurements of their absorption in UV range. The HPTLC determination was performed by modifying the solvent system developed for TLC and then scanning the spots by densitometry.

IN continuation of our earlier work on the analysis of various analgesic preparations in various combinations by quantitative TLC, we have examined a drug combination comprising of propyphenazone and ketoprofen.

The combination of propyphenazone and ketoprofen is a widely marketed preparation for analgesic, antipyretic and antiinflammatory actions.[1] Determination of propyphenazone in a combination preparation containing phenacetin, caffeine and persedon has been achieved by TLC, UV and GC[2] whereas another combination with caffeine and paracetamol has been analysed by HPLC[3] and GC[4]. Individual assay procedures on ketoprofen utilizing TLC, HPLC, HPTLC, UV spectroscopy, potentiometric titrations, GC, colorimetry have been described in the literature.[5-13] An analysis of a metabolite of ketoprofen in the horse using GCMS and HPLV has also been reported.[4] A BP method for the assay of ketoprofen involving titrimetry is also available.[15] In the present work we have performed a simultaneous determination of propyphenazone and ketoprofen by quantitative TLC and HPTLC as there are no reports of their analysis in the form of the present formulations.

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EXPERIMENTAL

MATERIALS

The pharmacopoeial grade propyphenazone and ketoprofen were employed as standards and procured form M/s Juggat pharmaceuticals, Bangalore. All reagents used are of AR grade.

Developing Solvent

Solvent system used in TLC technique consisted of a mixture of butyl acetate : chloroform : formic acid : benzene : toluene (60:20:20:10:10). For HPTLC, the solvent system was modified to a less polar solvent system. It comprised of butyl acetate: chloroform : formic acid : benzene : toluene (16:8:4:4:6).

Standard curve by quantitative TLC

Ketoprofen solutions in concentration of 0.4, 0.6, 0.8, 1.0, 1.2 1.4 and 1.6 mg/ml were prepared in methanol. Each of these solutions were spotted as 0.1 ml in duplicate on activated TLC plates (20 X 20 cm) coated with silica-gel G of thickness 0.5mm. The chromatograms were developed in the solvent

system contained in a closed chromatographic chamber by ascending technique. The chromatographic spots (Rf = 0.84) were detected by spraying with spraying reagent. The plates were heated at 80° C and gave brown coloured spots. The spots were scraped and collected in dry clean centrifuge tubes, containing 10 ml of methanol. The solutions were filtered and the absorbances of these solutions with respect to the blank (extracted by same method) were measured at 255nm using Jasco model MHT-344 autoscan UV Visible spectrophotometer. The duplicate uncoloured spots which were not sprayed were also scraped, centrifuged after adding 10ml methanol and the supernatant was used for taking the readings of both pure standard and the sample. Blank samples were prepared by scraping of silica Gel G and by processing it exactly in the same manner as the sample.

Using a similar procedure, the samples of propyphenazone were prepared in the concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 1.4 and 1.8 mg/ml in methanol. Brown coloured spots (Rf = 0.56) were obtained by spraying with 0.1N Iodine solution. The uncoloured duplicate spots of propyphenazone were also scraped and extracted with 10 ml of methanol and their absorbances were measured at 245 nm with respect to the blank.

Form the absorbance values, the standard curves for propyphenazone and ketoprofen were prepared and are shown in **Fig.1**

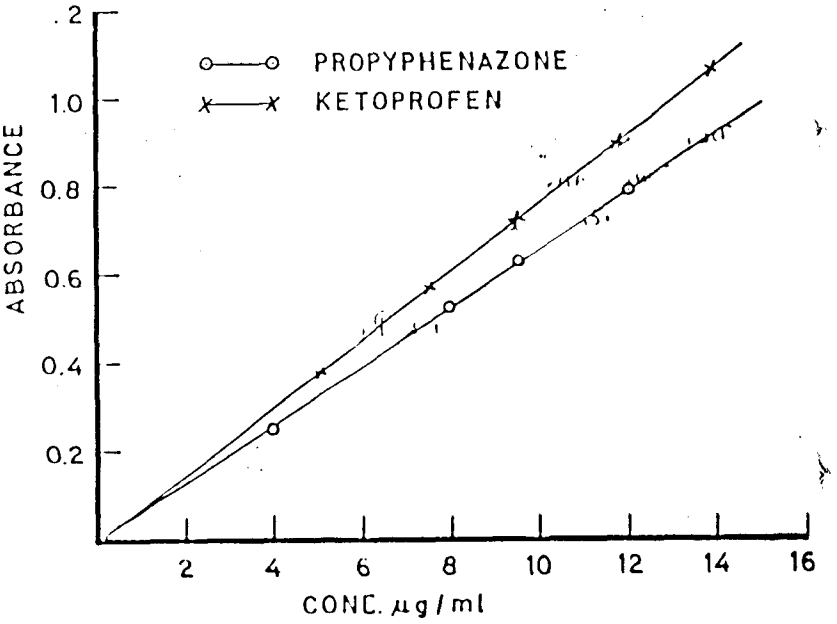

FIG.1. STANDARD CURVES FOR QUANTITATIVE TLC

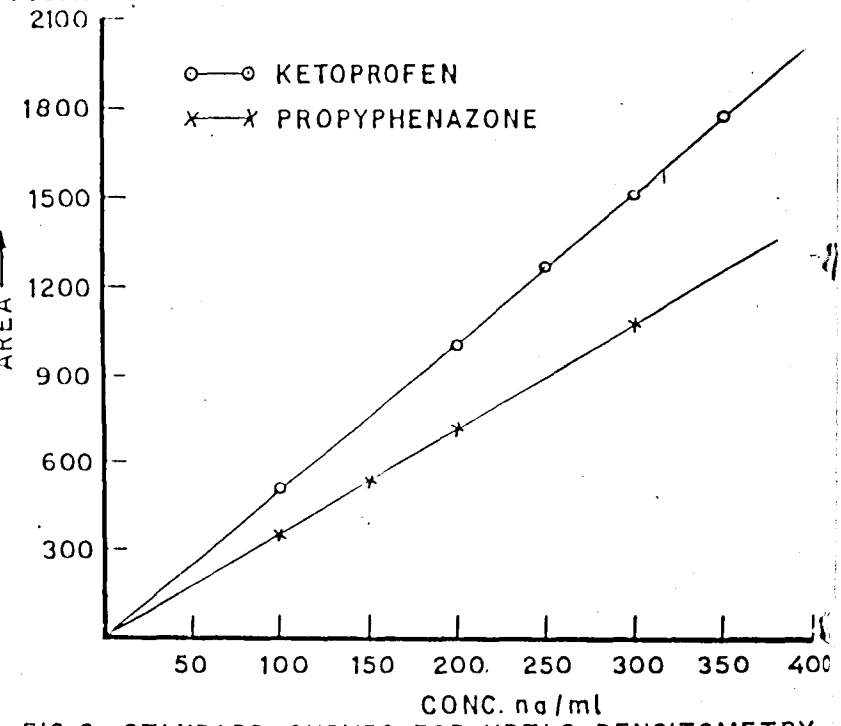

FIG.2. STANDARD CURVES FOR HPTLC DENSITOMETRY

ASSAY PREPARATION BY QUANTITATIVE TLC

Ten capsules were accurately weighed and the contents were finely powdered. The powder equivalent to the average weight was treated with 10 ml of methanol and the volume was made upto 100 ml with ethanol. The solutions were filtered to obtain a clear solution. The spotting, development, detection and extraction of the separated constituents were done in the same manner as described for the pure drug samples. The absorbance of the solutions was measured at 245nm and 255nm for propyphenazone and ketoprofen respectively. The results of the estimation and recovery studies are given in **Table 1.**

Standard curve by HPTLC Densitometry

Propyphenazone and ketoprofen solutions were prepared in the concentrations of 1 mg/ml in methanol separately and further diluted to give concen-

Table I: Recovery Data for the Assay by Quantitative TLC

Formulation	Labelled amount (mg/cap.)	Amount added (mg/cap.)	Amount found (mg/cap.)*	%Recovery	CV%
Brand A					
Propyphenazone	150	150	148.5	98.40	0.001
Ketoprofen	50	50	49.2	99.44	0.002

*Each value is an average of five determinations

Table II: Recovery for the Assay Data by HPTLC Densitometry

Formulation	Labelled Amount (mg/cap.)	Amount Added (mg)	Amount Found (mg/cap.)	% Recovery	CV%**
Brand A					
Propyphenazone	150	150	150.863	100.57	1.95
Ketoprofen	50	50	49.960	99.92	4.89

*Each value is an average of five determinations

** Coefficient of varience (%) = $\frac{\text{height}}{\text{area}}$

trations of 50 ng/µl. One ml of each of these pure solutions were mixed together and amounts of 2,3,4,5,6 and 7 µl were spotted band wise using linomat IV, automatic sampler on HPTLC plates (10 X 10 cm) coated with silica gel (60 F 254) of thickness 200 µm. The chromatograms were then developed in the earlier mentioned solvent system contained in a closed chromatographic chamber (10 x 12 x 4 inch) lined with Whatman filter paper No. 1 with glass-lid by ascending technique. The developed spots (Rf of propyphenazone = 0.52 and ketoprofen =0.73) were blow dried and scanned in a Camag TLC scanner II at 265 nm for absorbance by reflectance. The spot areas were evaluated and a plot of area vs concentration was obtained by the camag cats software program (**Fig 2**).

Assay procedure by HPTLC Densitometry

Ten capsules were accurately weighed and powder equivalent to the average weight was extracted with 25ml of methanol. The solution was centrifuged and 2.0 µl quantity was spotted. The development, detection and extraction were done in the same manner as described for the pure drugs. The absorbance of the solutions was measured at 265nm on a Camag scanner. The results of the estimation are shown in **Table 2**.

RESULTS & DISCUSSION

The proposed methods should become very valuable in the routine analysis of the present combination formulation. The HPTLC densitometry has

the added advantage of high efficiency sensitivity and requires small sample amounts. A comparison of the analytical data obtained from the quantitative TLC and HPTLC was also done by performing the f-test (for propyphenazone it is 1.617 and for ketoprofen it is 2.85) and t-test (propyphenazone corresponds to 0.914 and for ketoprofen it is 2.876). It was found that there was very small variation in the final results.

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REFERENCES

1. Martindale, The extra pharmacopoeia, 30th edition, Royal Pharmaceutical society of Great Britain, 1993, 21.3, 31.3.
2. Gary G. Liversidge, Analytical Profits of Drug substances, N.Y., 1981, 10, 443-471.
3. British Pharmacopoeia, her Majesty's Stationary Office, London, 1980, Addendum 1982, 69, 130.
4. Dobbis, M.F. and Touchstone, J.C., Practice of Thin Layer chromatography, John Wiley and Sons, N.Y., 1983, 1-13, 233, 339- 358.
5. Sharma J. & Fried B., Hand Book of thin layer chromatography, 1991, 20, 26.
6. Benoit. E., Jaussaund, P., Besse. S., Videmann. B., Courtot. D. and Delatour. P., Journal of chromatography & Biomedical applications, 1992, 583, 167-173.
7. Emmanual. J., Fernandes, N.N., **The Eastern Pharmacist**, June 1988, 139-140.
8. Hoefelfinger. P., Schmidli. B. and Kitter. H., Arch Phar., 1964, 297, 641-648.
9. Mamolo. M.G., Vio. L.,**J. Pharm. Biomed. Anal.**, 1985, 3, 157-164.
10. Sioufi.A., Sandrevan. N. and Pommier. F., **J. Chromatogr. Biomed. Appli.**, 1980, 211, 377-381.
11. Markovic. S., Kusec. Z., **Pharmazie**, 1990, 45, 935-936.
12. Jefferies. T.M., Thomas. W.O.A., Partitt. R.T., **J. Chromatogr. Biomed. Appli.**, 1979, 162, 122-124.
13. Rossetti. V., Lombard. A., Buffa. H.,**J. Pharm. Biomed. Appli.**, 1986, 4, 673-676.
14. Sallustio.B.C., Abas. A., Hayball.P.,**J. Chromatogr. Biomed. Appli.**, 1986, 374, 329-337.
15. Menzel - Soglowek. S., Geisslinger. G., Brune. K., **J. Chromatogr. Biomed. Appli.**, 1990 532, 295-303.
16. Hayball. P.J., Nation. R.L., Bochner. F., Leu.R.K., **J. Chromatogr. Biomed. Appli.**, 1991, 570, 446-452.
17. Oda. T., Asakawa.N., Abe.S., Yoshida.T., Sato.T., **J. Chromatogr. Biomed. Appli.**, 1991, 572, 133-141.