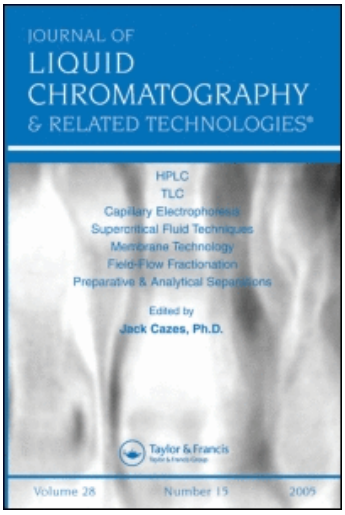

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Determination and Quantitation of Bendroflumethiazide and Its Degradation Products Using HPLC

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DETERMINATION AND QUANTITATION OF BENDROFLUMETHIAZIDE AND ITS DEGRADATION PRODUCTS USING HPLC

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ABSTRACT

A method for the determination and quantitation of bendroflumethiazide (BFMT) and its degradation products hydroflumethiazide (HFMT) and 5-trifluoromethyl-2,4-disulphamoylaniline (TFSA) using RP-HPLC is described. Good reproducibility and accuracy of the assay can be achieved without the use of a buffer solution in the mobile phase. A pH of 2.0 of the solvent and the use of dark flasks will be important to give a good stability of the samples during automatic injection. The limits of detection for BFMT, HFMT and TFSA were respectively 1.3, 0.8 and 0.7 µg/ml (α = 0.05).

INTRODUCTION

Bendroflumethiazide (BFMT) (3-benzyl-3,4-dihydro-6-trifluoromethyl-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide), [CAS 73-48-3], is a potent diuretic drug mentioned in the US (1), British (2), German (3) and other pharmacopoeias.

Several methods have been reported for the determination of BFMT and its degradation product 5-trifluoromethyl-2,4-disulphamoylaniline (TFSA) using

TLC (2-6). GC (7,8) and HPLC with either fluorescence (9) or UV detection (1,10-15). Perlman and Kirschbaum report on problems with the solvent used for the samples resulting either in enhanced peak response or in lack of stability (14,12,16). The last point will be very important if samples are to be injected automatically by an autosampler.

A recent study (17) shows that BFMT under certain conditions may produce hydroflumethiazide (HFMT) (3,4-dihydro-6-trifluoromethyl-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide).

The aim of this report is to describe a rapid, accurate and precise HPLC method for the simultaneous determination and quantitation of BFMT, HFMT and TFSA applicable to injection by an autosampler.

EXPERIMENTAL

Chemicals and Reagents

BFMT was kindly provided by Glaxo (Glaxo Group Research, Greenford, Middlesex, GB), HFMT was supplied by Sigma Chemicals (St.Louis, Missouri, USA) and both were used as obtained. TFSA was prepared by hydrolysis of BFMT as described elsewhere (11). Its purity was confirmed by m.p., IR and HPLC assay using the British Pharmacopoeia CRS as standard and was found to be 99.6 %. Methanol (E.Merck,Darmstadt, Germany, Art.Nr.6009) contained not more than 0.001 % formaldehyde and was found to give no interactions with BFMT (16). All other reagents were analytical or HPLC grade and were used as obtained.

Instrumentation and Chromatographic Conditions

The system was equipped with a Spectroflow 400 pump (Kratos Analytical, Ramsey, New Jersey, USA), a Marathon autosampler (Spark Holland, Emmen, The Netherlands) with a Rheodyne 7010 injection valve and a 50 µl loop, a BT 8200 UV detector (Biotronik GmbH, Maintal, Germany), a DT 2801 A/D converter (Data Translation, Locke Drive, MA, USA) and a PC 16/16

computer (Siemens AG, Fürth, Germany). The software CHROM+ Ver.2.04 (Laboratory-Technologies Corp., Wilmington, MA, USA) was supported by a self-written BASIC program.

The column was 300 x 4 mm i.d. with a guard column 5 x 4 mm i.d., both packed with Nucleosil-100 Phenyl of particle size 7 µm (Knauer GmbH, Berlin, Germany).

The solvent for the samples was prepared by dissolving 750 mg of KCl in 10 ml of 1N HCl and about 400 ml of distilled water in a 1000 ml volumetric flask, adding 400 ml of methanol, mixing under cooling and diluting with water to volume (MeOH / buffer 40% v/v). The pH was ~ 2.0, within the limits of 1.95 and 2.05. Only low activity glass was used and the samples were kept in dark flasks.

The mobile phase was prepared similarly by diluting to volume 400 ml of methanol with distilled water in a 1000 ml volumetric flask under cooling, mixing and sonificating for 15 min.

The flow rate was 1.5 ml/min (≈ 20 MPa), the oven temperature was set to 35 °C and the detector was set to λ = 270 nm and AUFS = 0.005. The peak areas as integrated by the Chrom+ software were used as a quantitative measure.

System Suitability and Calibration

The dead volume of the system was determined by repeated injection of solutions of 8 µg/ml sodium nitrate in MeOH / H_2O 40% v/v and detection at λ = 220 nm.

The system suitability test of the US pharmacopeia was performed with a solution containing 50.9 µg/ml BFMT, 27.4 µg/ml HFMT and 24.2 µg/ml TFSA (n = 12).

For the calibration triplicate and randomized injections of 7 solutions of BFMT (0.9 to 105.5 µg/ml), HFMT (0.6 to 71.0 µg/ml) and TFSA (0.6 to 69.9 µg/ml) were performed at two days. A linear calibration curve was fitted by linear regression to the means of the peak areas (n = 14).

The accuracy of the assay was investigated at two levels of BFMT, HFMT and TFSA of about 90, 2 and 2 µg/ml (a) and about 45, 12 and 12 µg/ml (b) respectively. For each level two solutions were prepared at three days (n = 6) and injected in triplicate.

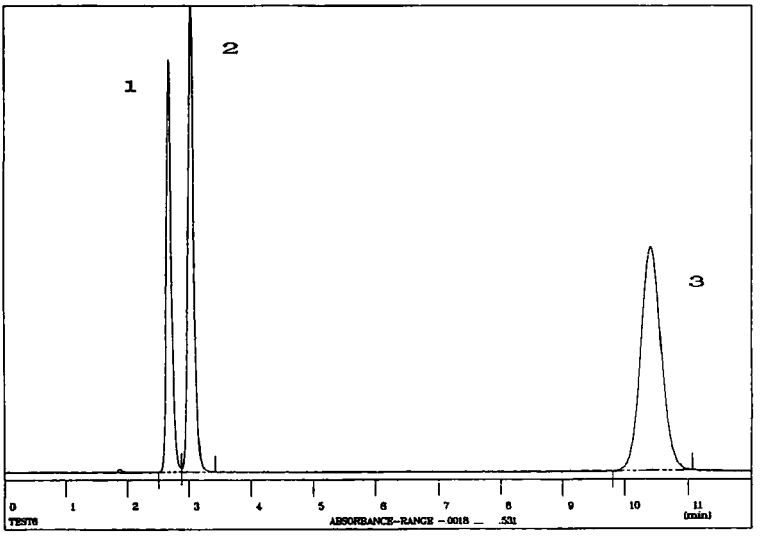

FIGURE 1 Chromatogram of BFMT and its Degradation Compounds.
Mobile Phase MeOH/Water 40% v/v, 1.5 ml/min.Detection UV 270 nm
1 = TFSA, 2 = HFMT, 3 = BFMT

RESULTS AND DISCUSSION

A typical chromatogram of BFMT and its degradation compounds is shown in **Figure 1**. The retention times are summarized in **Table 1** together with the capacity factors k' and the tailing factors (all data recorded at different days).
t_0 was found to be 1.213 ± 0.006 min (n = 11).

We found linearity of the peak area responses over the whole range tested as well as no significant difference between the slopes at different days (α = 0.05) and between intercept and zero (α = 0.1). Homogeneity of the variances of the peak areas of triplicate injections was given over the tested range (Cochran test, α = 0.05). Thus the reproducibility was calculated as the mean standard deviation (S.D.) of triplicate injections of 24 samples (BFMT n = 23) and found to be not different from that of 12 injections of the same sample (USP XXII system suitability test). At both levels recovery was satisfactory. The limits of the assay (α = 0.05) were

TABLE 1
Retention Times, Capacity Factors and Tailings Factors for TFSA, HFMT and BFMT (Mean ± S.D). For Ret.Time and k' n = 55, for Tailing Factor n = 48

Compound	Retention Time [min]	k'	Tailing factor
TFSA	2.668 ± 0.008	1.20	1.34 ± 0.05
HFMT	3.030 ± 0.010	1.50	1.27 ± 0.04
BFMT	10.434 ± 0.095	7.60	1.07 ± 0.01

calculated as described by Montag (18) . **Table 2** summarizes the results for each compound.

It is important for the performance of a stability indicating assay to focus the attention on two different problems, i.e. the composition of the mobile phase and of the solvent for the samples.

Only Hassan (11) suggests a mobile phase without buffer solutions, but this method is not able to separate TFSA from HFMT. The USP XXII (1) and other authors (12,14) use an acetate buffer with sodium chloride in the mobile phase. We did not find this buffer or any other to improve the performance and thus preferred a methanol/water system, which is less toxic than acetonitrile.

Usually the solvent of the samples should be very similar to the mobile phase (**Figure 2 A**), but methanol was proposed by some authors (1,11,12). However great problems can result if the solvent of the sample is methanol. A broadening or a splitting of the peaks was described elsewhere and attributed to intramolecular hydrogen bonds (14,16). **Figure 2 B** shows a very bizzare chromatogram of 103, 44 and 66 µg/ml BFMT, HFMT and TFSA respectively in methanol resulting from an analysis which was performed exactly as described in the USP XXII (1) without any time delay.

Changing the solvent from methanol to mobile phase will cause a lack of stability of BFMT, which undergoes a very rapid hydrolysis expecially by light stress (12), and therefore will not be suitable for long stand-by times, e.g. during

TABLE 2
Reproducibility and Accuracy of the Assay, Limits of Detection and Quantitation. For Details s.Text.

	BFMT	HFMT	TFSA
Reproducibility [µg/ml]	0.44	0.14	0.11
Accuracy [%] ± S.D. (a)	100.3 ± 1.3	99.2 ± 5.6	103.7 ± 4.8
(b)	100.0 ± 1.8	102.2 ± 2.8	101.4 ± 2.4
Limit of Detection [µg/ml]	1.3	0.8	0.7
[µmol/l]	2.5	2.5	2.1
Limit of Quantitation [µg/ml]	2.4	1.6	1.4
[µmol/l]	5.0	4.9	4.4

automatic injection. As is well known (19), a pH of 2.0 will decrease the rate of decomposition. We confirmed these results for normal laboratory light exposure and for room temperature we found an apparent first order rate constant $^1k = 4.0 * 10^{-6}$ [min^{-1}] which causes a decomposition of BFMT of about 0.3 mol% within 12 h. Under the conditions of the USP XXII (1) a pH of 5.2 results when the mobile phase is used as solvent. For this pH we found a constant $^1k = 6.0 * 10^{-5}$ [min^{-1}] and consequently a degradation of about 4.2 mol% within 12 h. Table 3 highlights this very clearly. If the solvent is not buffered to pH 2.0, the changes of the areas after 24 h for BFMT and TFSA are unacceptable. Only HFMT appears to be sufficiently stable within 24 h, but this may be due to a faster reverse reaction of TFSA with formaldehyde compared to that with phenylacetaldehyde.

When the sample was stored in a dark flask, no detectable degradation was found for BFMT after 24 h. Figure 3 shows the chromatogram of a solution of 109.0 µg/ml BFMT either in methanol / water or in methanol / buffer pH 2.0 and its decomposition in a dark flask after 24 h at room temperature.

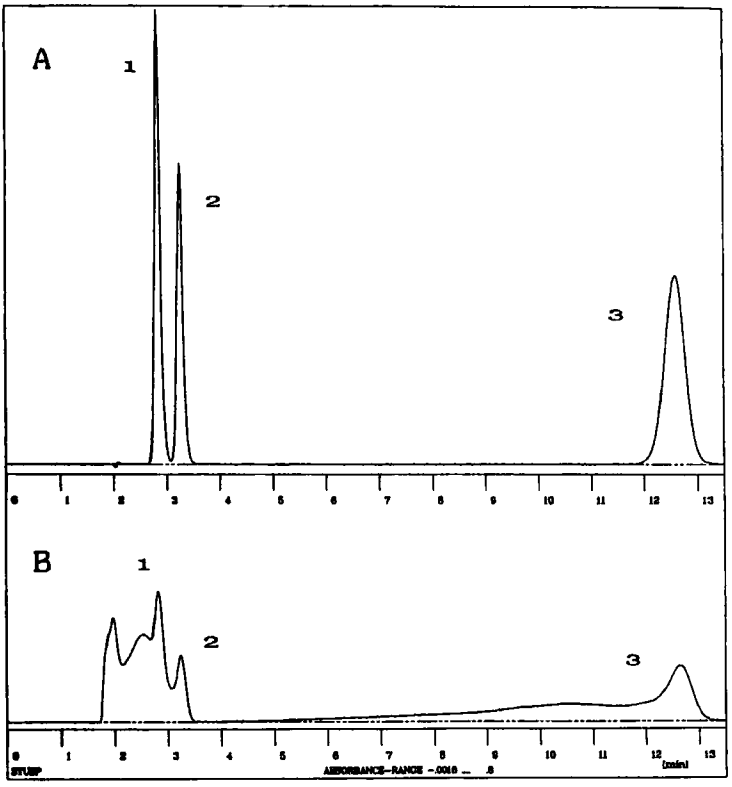

FIGURE 2 Effect of the Solvent. Chromatographic Conditions of the USP XXII
1 = TFSA, 2 = HFMT, 3 = BFMT
A Solvent mobile phase, B Solvent Methanol

If an internal standard is reasonable in case of complicated extraction e.g. from tablets, we found salicylamide - as proposed by Hassan (11) - to be useful, since it is eluted between HFMT and BFMT.

CONCLUSIONS

The very simple RP-HPLC method reported here is suitable for the determination and quantitation of BFMT in bulk or in dosage forms and by

TABLE 3
Influence of pH on the Stability at Normal Laboratory Light Exposure.
Solution 1 = 56.8, 30.2 and 30.0 µg/ml BFMT, HFMT and TFSA respectively.
Solution 2 = 50.9, 27.4 and 24.2 µg/ml BFMT, HFMT and TFSA respectively
Peak-Areas [Volt*s], Mean ± S.D. (n = 3)

Solvent	1 MeOH / H_2O 40% v/v			2 MeOH / Buffer pH 2.0 40% v/v		
Time [h]	BFMT	HFMT	TFSA	BFMT	HFMT	TFSA
0	7.285 ± 0.020	3.668 ± 0.030	3.595 ± 0.022	5.728 ± 0.030	3.484 ± 0.010	2.736 ± 0.010
24	4.990 ± 0.018	3.637 ± 0.011	5.438 ± 0.015	5.707 ± 0.113	3.550 ± 0.010	2.796 ± 0.003

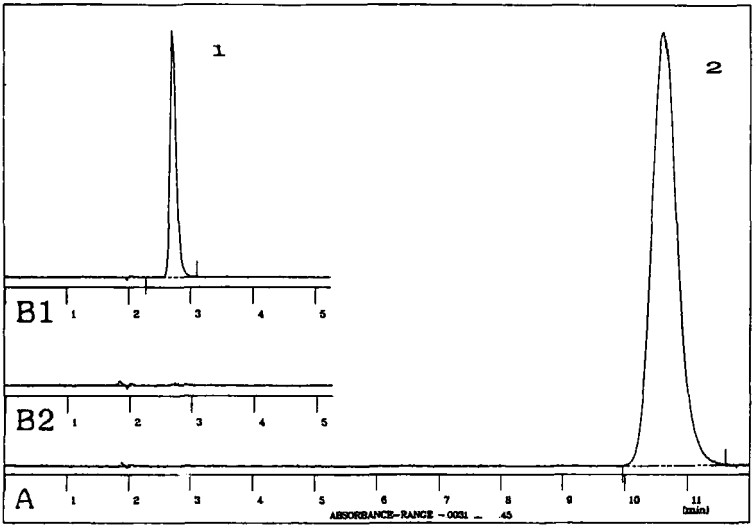

FIGURE 3 Stability of BFMT in Dark Flasks during Automatic Injection.
Chromatogram of 109 µg/ml BFMT in MeOH / Water or
MeOH / Buffer pH 2.0 40% v/v. All Graphs with same Ordinate Scale.
1 = TFSA, 2 = BFMT
A Time = 0; B1 time = 24 h, not buffered, B2 time = 24 h, buffered

automatic injection with long stand-by times up to about 12 h. For the standard dose of 5 mg BFMT, the detection of 0.2 mol% degradation will be achieved by dissolving the sample in 10 ml. The sensitivity of this method thus lays below the limits of any pharmacopoeia (1.5 and 1.0 % respectively for the USP (1) and BP (2)).

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REFERENCES

(1) U.S.Pharmacopoeia XXII, U.S. Pharmacopeial Convention Inc., Rockville MD, 1989, p.144

(2) British Pharmacopoeia, Her Majesty's Stationary Office, London, 1988, p.60

(3) Deutsches Arzneibuch 9.Ausgabe, Deutscher Apotheker Verlag, Stuttgart, 1986, p.554

(4) Hennig,U.G., Moskalyk,R.E., and Chatten,L.G., Semiaqueous Potentiometric Determinations of Apparent pKa_t Values for Benzothiadiazines and Detection of Decomposition during Solubility Variation with pH Studies, J.Pharm.Sci., 70,317, 1981

(5) Schäfer-Korting,M., and Mutschler, E., Pharmacokinetics of Bendroflumethiazide Alone and in Combination with Hydralazine, Eur.J.Clin.Pharmacol., 21, 315, 1982

(6) Agarwal,S.P., and Nwaiwu,J., Detection of diuretic and oral hypoglycemic drugs on thin-layer plates using π-acceptors as spray reagent, J.Chromatog., 351, 383, 1986

(7) Beermann,B., Groschinsky-Grind,M., and Lindström,B., A GLC Assay for Bendroflumethiazide.Preliminary Data about Its Plasma Level in Man, Eur.J.Clin.Pharmacol., 10, 293, 1976

(8) Fagerlund,C., Hartvig,P., and Lindström,B., Extraction Alkylation of Sulfonamide Diuretics and their Determination by Electron Capture Gas Chromatography, J.Chromatog., 168, 107, 1979

(9) Hikal,A.H., Determination of bendroflumethiazide in plasma by high performance liquid chromatography and fluorescence detection, J.Liq.Chromatog., 9, 2937, 1986

(10) Moskalyk, R.E., Locock R.A., Chatten L.G., Veltman A.M., and Bielech M.F., Determination of Polythiazide in Pharmaceutical Dosage Forms by High Pressure Liquid Chromatography, J.Pharm.Sci, 64, 1406, 1975

(11) Hassan,S.M., A Stability-Indicating Assay for Bendrofluazide Using High-Performance Liquid Chromatography, Chromatogr., 17, 101, 1983

(12) Perlman,S., Szyper,M., and Kirschbaum,J.J., High-Performance Liquid Chromatographic Analysis of Nadolol and Bendroflumethiazide Combination Tablet Formulations, J.Pharm.Sci., 73, 259, 1984

(13) Croo,F.de, Bossche,W.van den, and Moerloose,P.de, High-performance liquid chromatographic behaviour of some pharmaceutically important thiazide, loop and potassium-sparing diuretics, J.Chromatog., 325, 395, 1985

(14) Perlman,S., and Kirschbaum,J.J., Enhanced peak responses due to solvent interactions in high-performance liquid chromatography, J.Chromatog., 357, 39, 1986

(15) Smith,R.M., Murilla,G.A., and Hurdley,T.G., Retention reproducibility of thiazide diuretics and related drugs in reversed-phase high-performance liquid chromatography, J.Chromatog., 384, 259, 1987

(16) Kirschbaum,J., Perlman,S., and Poet,R.B., Anomalies in HPLC, J.Chromatog.Sci, 20, 336, 1982

(17) Frontini,R., Einfluss von wasserlöslichen Polymeren auf die Stabilität von Bendroflumethiazid in festen Dispersionen, thesis university of Hamburg, in preparation

(18) Montag A., Beitrag zur Ermittlung der Nachweis- und Bestimmungsgrenze analytischer Meßverfahren, Anal.Chem., 312, 96, 1982

(19) Mollica,J.A., Rehm,C.R., Smith,J.B., and Govan,H.K., Hydrolysis of Benzothiadiazines, J.Pharm.Sci., 60, 1380, 1971