Analysis of Doping Substances by Gas Chromatography with Mass Selectivity Detector

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Summary: An analytical method for doping control analysis has been studied. Twenty nine drugs including eighteen narcotic analgesic, nine β-blockers, one stimulant and one standard were analyzed simultaneously by gas chromatography equipped with nitrogen phosphorous detector and gas chromatography combined with mass selective detector after derivatization of the drugs.

Introduction

Since Beckett [1] first applied sensitive gas chromatographic testing procedures to identify abuse drugs at an athletic event in 1965 and since then many procedures for the screening of stimulants and opioids have been introduced [2-6]. Gas chromatography combined with mass spectrometer (GS-MS) has been widely used for the confirmation of the results obtained by screening procedures. Gattin *et al.* [7] have used gas chromatography for the screening of opioids where the urine extracts were analyzed after derivatization by trifluoroacetic anhydride. Maurer *et al.* [8] have analyzed opioids with GC-MS after acetylation of the samples. Fang *et al.* [9] have compared different derivatization for twelve narcotic analgesics and two stimulants with *N*-methyl-*N*-trimethylsilyltrifluoro-acetamide, *N*-methyl-bis(trifluoroacetamide) and trifluoroacetic anhydride. In routine analysis for doping control during any sporting event and the demand from the International sporting committee to have a fair competition from the participant, a method for screening narcotic analgesics, β-blocker and various stimulants becomes an essential part of these sporting events. Because more than twenty drugs with different chemical properties are to be detected simultaneously, it is necessary to develope a suitable derivatization procedure and separation conditions by chromatographic technique. In this study we report a method for the simultaneous separation and detection of eighteen narcotic analgesics, nine β-blockers, one stimulant and one standard by gas chromatography with a nitrogen-phosphorous detector (GC-NPD) and with a mass selective detector (GC-MSD).

Results and Discussion

Separation of the drugs

Retention times of the drugs obtained by GC-NPD and GC-MSD analyses are shown in Table 1 and 2. Characteristic ions of mass spectra of the compounds are shown in Table 2. Total ion current obtained from the analysis of the sample prepared as described under derivatization procedure is shown in Fig.1. All drugs yielded chromatographic peaks. As shown in Fig. 1 and Table 1, most gas chromatographic peaks of the drugs are well separated by GC-NPD and GC-MSD. Although in the chromatogram obtained by GC-NPD, the peaks of propranolol overlapped that of atenolol and acebutalol overlapped that of phenazocine, it is easy to identify them with their characteristic ions. The retention times obtained by GC-NPD were shorter than those by GC-MSD because the column used in GC-NPD was shorter.

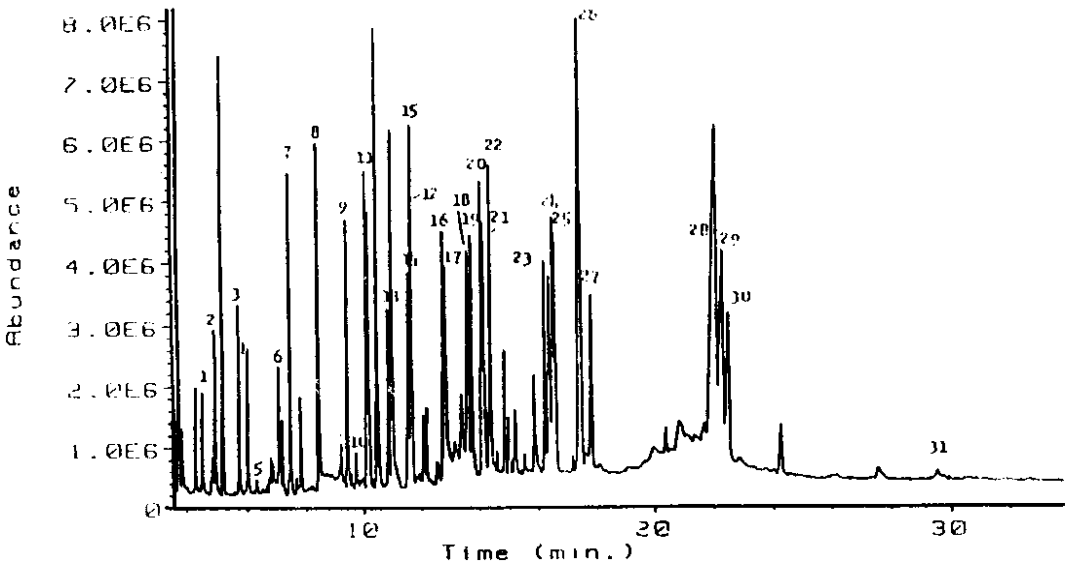

Fig.1: Total ion current obtained from the GC-MSD analysis of a sample containing 29 standards prepared as described in the derivatization procedure. The peak numbers in the figure are same as those given in Table 2

Table 1: GC-NPD data for TMS-TFA derivatives of narcotic analgesics and β-blockers

Compound Name	Retention time (min)	Relative Retention time (min)
Pethidine (PT)	3.079	0.4690
Trimeperidine (TR)	3.657	0.5570
Etholheptazine (EH)	3.850	0.5864
Dextropropoxyphene (PP)	4.079	0.6213
	6.332	1.0482
*Ethamivan (ET)	4.639	0.7066
Atprenolol (Alp)	4.948	0.7537
Oxprenotol (Oxp)	5.700	0.8682
Methadone (ME)	6.378	0.9715
Methaquatone (I.S)	6.565	1.0000
Leverphanol (LE)	7.076	1.0778
Metoprolol (Met)	7.221	1.0999
Pentazocine (PE)	7.779	1.1849
Propranolol (Pro	8.237	1.2547
Atenolol (Ate)	8.237	1.2547
Dihydrocodeine (DH)	9.106	1.3871
Sotatol (Sot)	9.235	1.4067
Nadolol (Nad)	10.025	1.5270
Codeine (CD)	10.163	1.5481
Dipipanone (DI)	10.358	1.5481
Ethylmorphine (EM)	10.492	1.5982
Morphine (MO)	10.751	1.6376
Heroin (HE)	12.823	1.9532
Acebutolol (Ace)	13.199	1.9983
Phenazocine (PH)	13.119	1.9983
Labetalol (Lab)	13.434	2.0463
	13.869	2.1126
Anileridine (AN)	15.544	2.3677
Dextromoramide (MR)	16.214	2.4698
Natbuphine (NA	16.404	2.4987
Buprenorphine (BN)	19.189	3.9729

* : stimulant.

Derivatization conditions

Derivatization reaction was affected by many factors such as the temperature of the reaction and the amounts of the derivatizing reagents. In this procedure, MSTFA and MBTFA were used as the derivatizing reagents. Different temperatures were tried for the reaction and it was found that four peaks were obtained for labetalol when 80°C was used while two peaks were obtained at 60°C. These two peaks at the lower temperature corresponded to the two isomers of labetalol. For complete derivatization of all drugs in a sample 2 ml of MSTFA and 600 µl of MBTFA were sufficient however, 100 µl of MSTFA and 30 µl of MBTFA were enough if a sample contained three or four drugs or the extract of each urine sample.

Multiple chromatographic peaks

Most drugs yielded a single chromatogra-phic peak under present derivatization conditions, however, some drugs such as nalbuphine, methodone, sotalol and buprenorphine yielded multiple peaks. This might be caused by the side reactions or incompleteness of the derivatization. Nalbuphine yielded two peaks at 13.299 and 16.404 min. in GC-NPD and 17.427 and 22.511 min. by GC-MSD. The chromatographic peak of longer

Table 2: GC-MSD data for TMS-TFA derivatives of narcotic analgesic and β-blockers

Compound Name	Mol. Wt.	Peak No.	Retention time (min)	Characteristic ions
Pethidine	247	2	4.942	71, 172, 247 (M^+)
Trimeperidine	275	3	5.746	186, 275, (M^+)
Ethoheptazine	261	4	6.027	57, 188, 261 (M^+)
Dextropropoxyphene	339	1	4.527	115, 208, 91
		5	6.334	58, 91, 178
*Ethamivan	223	6	7.074	223, 295 (M^+), 193
Alprenolol	249	7	7.469	284, 129, 402
Oxprenolol	265	8	8.456	284, 129, 418
Methadone	309	9	9.449	72, 294
Methaqualone	250	10	9.739	235, 250 (M^+)
Levorphanol	257	11	10.117	59, 329 (M^+), 150
Metoprolol	267	12	10.207	284, 129, 420
Pentazocine	285	13	10.857	289, 342, 357 (M^+)
Atenolol	266	14	11.551	284, 158, 359
Propranolol	259	15	11.674	284, 129, 247 (M^+)
Sotalol	272	16	12.774	344, 497
Dihydrocodeine	301	17	12.845	373 (M^+), 146, 236
Nadolol	309	18	13.620	86, 510, 409
Codeine	299	19	13.751	371 (M^+), 178,234
Dipipanone	349	20	14.086	112, 334 (M^+-15)
Ethylmorphine	313	21	14.166	385 (M^+), 192, 234
Morphine	285	22	14.399	73, 236, 429 (M^+)
Heroin	369	23	16.390	327, 369 (M^+), 268
Acebutolol	336	24	16.516	234, 129
Phenazocine	321	25	16.586	302, 378
Labetalol	328	26	17.427	292
		27	17.827	292
Anilieridine	352	28	22.071	246, 91, 115
Dextromoramide	392	29	22.302	100, 265, 306
Nalbuphine	357	30	22.511	73, 573 (M^+), 428
Buprenorphine	467	31	29.510	55, 189, 273
		32	40.951	450, 55, 482

*The base peak ions are shown in first in the characteristic ions.

retention time was used for the identifi-cation of the compound because M^+ ion (573) has been obtained in its mass spectrum. Two peaks were obtained from methadone by GC-NPD at 6.378 and 7.221 min. and by GC-MSD at 9.449 and 10.481 min. The first one was the main product of the derivatization, the ion 294 (M^+ 15) has been obtained in the spectrum of the peak. Sotalol yielded two peaks also in GC-NPD at (9.235 and 9.643 min.) and in GC-MSD (12.744 and 13.387 min.). The first was the main product of the derivatization, its mass spectrum showed ion of 344, the M^+ ion of sotalol-TMS. A small peak at 19.189 min. in GC-NPD and 29.510 min. in GC-MSD was obtained from buprenorphine followed by a big peak at 26.079 min. in GC-NPD and 40.951 min. in GC-MSD (not shown in Fig.1). The second peak was 17 times as high as that of the first peak in GC-NPD and about 3.3 times in GC-MSD. Both peaks were used for the identification of the compound.

The decomposition of dextropropoxyphene was observed in GC-NPD and GC-MSD analyses. Two peaks were obtained from the compound at 4.079 and 6.882 min. in GC-NPD and 4.527 and 6.334 min. in GC-MSD. The peak at 4.079 min. in GC-NPD corresponded to that at 6.334 min. in GC-MSD. It was found that decrease in temperature of the injection port to 180 °C increased the peak height at 6.882 min. in GC-NPD and at 5.334 min. in GC-MSD which indicates that this compound has a tendency to decomposes at the higher temperature. The detection limits of internal standard (methaqualone) using GC-NPD detection was 0.1 injected using split ratio 1:100 corresponded to 1 ng of compound.

In summary, GC-NPD analysis described in this study can be used for the screening of the drugs listed in Table 1, and GC-MSD could be a suporting technique for the confgirmation of the results obtained by GC-NPD. However, the presence of multiple chromatographic peaks obtained from some of the compounds does effect the detection limit of the drugs.

Experimental

Chemicals

Some of the drugs; pethidine, methadone hydrochloride, atenolol, propranolo, alprenolol, morphine hydrochloride, ethylmorphine, and methaqualone (internal standard) were kindly obtained from Professor Ingemar Bjorkhem of the Central Doping Lab., Huddinge Hospital. Codeine phosphate, ethamivan, levorphenol, phenazocine, acebutolol, labetolol, nadolol, oxrenolol and sotalol were obtained from Institute National de la Recherche Scientific (Canada). Anileridine-pentazocine, heroin, nalbupine, dextropropoxyphene, timeperidine, ethoheptazine, metoprolol, dihydrocodeine, dipipanone, dextromoramide and bupernophine were kindly provided with by

Professor Hans appelgren, Provincial Doping Lab. Swedish Agriculture University, Uppsala.

N-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Euro chemie (Oud-Beijerland, Holland) and *N*-methyl-bis (tri fluoroacetamide) (MBTFA) was obtained from Sigma Chemical Company St. Louis, MO (USA). All other chemicals were of analytical reagent grade and obtained from various commercial sources.

Derivatization of the samples

To a 5 ml test tube were added solutions containing 50-200 mg of each narcotic in Table 1. The mixture was evaporated to dryness under nitrogen gas. To the residue, 2 ml of MSTFA (1 mg/ml) were added. The solution was well vortexed and then heated at 70° C for 10 min in an oven. After that, 600 µl of MBTFA were added again and the solution was vortexed for a few min. and then heated at 70° C for further 10 min. The solution was then evaporated under nitrogen to a volume of 150 µl, 1 µl of the resulting solution was injected into GC-NPD and 2 µl into GC-MSD system.

Gas-chromatography with nitrogen phosphorous detector conditions (GC-NPD)

Gas chromatographic analysis was performed on a HP-5890A gas chromatograph combined with nitrogen phosphorous detector and HP-3393 integrator which controlled HP-5673A automatic sampler. A HP-5 capillary column was used (17 m x 0.2 mm i.d. with layer thickness 0.17 mm. All these components were supplied by Hewlett-Packard, Palo Alto CA, USA. Helium was used the carrier gas with a flow rate of 1 ml/min. with oven at 180° C. Flow rates of helium, air and hydrogen to the detector were 33.2, 109 and 3.4 ml/min. respectively. The temperature of the injection port was set at 250° C and that of the detector block at 280°C. The column temperature was set at 180°C for 1 min., then increased to 220° C at a rate of 10° C/min. the temperature was increased to 260° C at the rate of 5° C/min. and finally raised to 280° C/min. and kept there for 7 min. The split ratio was 1:100.

Gas chromatography mass selective detector conditions (GC-MSD)

The analysis was performed on a HP-5890A gas chromatograph combined with a HP-5970B mass selective detector which was equipped with HP-5 capillary column (25 m x 0.2 mm i.d. with layer thickness 0.17 m). Helium was used as the carrier gas with a flow rate of 0.98 ml/min. with oven set at 180° The column temperature program was similar to that of GC-NPD analysis except that at the beginning the temperature was increased without keeping it at 180° C for 1 min. and finally the column was kept at 28° C for 11 min. All other conditions were identical to GC-NPD.

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