Study on the doping control method for the detection of narcotic analgesics and β-blockers

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ABSTRACT The analytical method for doping control analysis has been studied. Twenty-nine drugs including eighteen narcotic analgesics, nine β-blockers, one stimulant and one internal standard were analysed simultaneously by gas chromatograph equipped with a nitrogen-phosphorous detector and gas chromatograph combined with a mass selective detector after derivatization of the drugs.

Key words Narcotic analgesics; β-Blockers; Doping control analysis

Since Beckett first applied sensitive gas chromatographic testing procedures to detect abuse drugs at an athletic event in 1965[1], many procedures for the screening of stimulants and opioids have been reported[2~5]. Gas chromatograph combined with mass spectrometer (GC-MS) has been widely used for the confirmation of the results obtained by screening procedures. Catlin et al[6] have used gas chromatography (GC) for the screening of opioids, the urine extracts were analysed after derivatization by trifluoroacetic anhydride. Maurer et al[7] have analysed opioids with GC-MS after acetylation of the samples. Fang et al have compared different derivatization procedures for twelve narcotic analgesics and two stimulants with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide, *N*-methyl-bis (trifluoroacetamide) and trifluoroacetic anhydride[8]. In the routine analysis for doping control during sport games, a method for the screening of narcotic analgesics and β-blockers is needed. Because more than twenty drugs with different chemical properties are to be detected simultaneously, it is necessary to find out a suitable derivatization procedure and gas chromatographic conditions. This paper presents a method for the simultaneous separation and detection of eighteen narcotic analgesics, nine β-blockers, one stimulant and one internal standard by gas chromatograph equipped with a nitrogen-phosphorous detector (GC-NPD) and gas chromatograph combined with a mass selective detector (GC-MSD).

EXPERIMENTAL

Standards and reagents

Pethidine was supplied by Qinghai Drug Factory; methadone hydrochloride and

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atenolol were obtained from Tianjin Central Drug Factory; propranolol and alprenolol were obtained from Shanghai Second Drug Factory; morphine hydrochloride was obtained from Huadong People's Drug Company; ethylmorphine was obtained from Beijing Medicine Company; methaqualone (internal standard) was obtained from Beijing Friendship Hospital, codeine phosphate, ethamivan, levorphanol, phenazocine, acebutolol, labetolol, nadolol, oxprenolol and sotalol were obtained from Institut National de la Recherche Scientifique (Canada); anileridine, pentazocine, heroin, nalbuphine, dextropropoxyphene, trimeperidine, ethoheptazine, metoprolol, dihydrocodeine, dipipanone, dextromoramide and buprenorphine were obtained from Zentrale Doping Kontroll-Labor des SMD (DDR); MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide) was obtained from Pierce (USA) and MBTFA [*N*-methylbis(trifluoroacetamide)] was from Sigma (USA). All other chemicals were of analytical reagent grade.

Derivatization of the samples

To a 5 ml test tube were added solutions containing 50 ~ 300 μg of narcotic analgesics, β-blockers, one stimulant and an internal standard listed in Table 1. The mixture was evaporated to dryness under nitrogen. To the residue 2 ml of MSTFA were added. The solution was well vortexed and then heated at 70 ℃ for 10 min in an oven. After that, 600 μl of MBTFA were added and the solution was vortexed for a few seconds and then heated at 70 ℃ for 10 min in an oven. 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 colinto GC-MSD.

Gas chromatograph-nitrogen phosphorous detector conditions

Gas chromatographic analysis was performed on a HP-5890A gas chromatograph combined with a nitrogen phosphorous detector (GC-NPD) and HP-3393 integrator which controlled a HP-7673A automatic sampler. A HP-5 capillary column was used (17 m × 0.2 mm ID). Helium was used as the carrier gas with a flow rate of 1.0 ml/min with oven at 180 ℃. 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 250 ℃ and that of the detector block was 280 ℃. The column temperature was set at 180 ℃ for 1 min, then increased to 220 ℃ at a rate of 10 ℃/min, after that the temperature was increased to 260 ℃ at a rate of 5 ℃/min, then to 280 ℃ at 10 ℃/min. Finally the column temperature was kept at 280 ℃ for 5 min.

Gas chromatograph-mass selective detector conditions

The gas chromatograph-mass selective detector (GC-MSD) analysis was performed on a HP-5890A gas chromatograph combined with a HP-5970B mass selective detector which was equipped with a HP-5 capillary column (25 m × 0.2 mm ID). Helium was used as the carrier gas with a flow rate of 0.98 ml/min with the oven at 180 ℃. The column temperature program was the same as that in GC-NPD analysis except that at the beginning the temperature was increased right away without staying at 180 ℃ for 1

min and finally the column was kept at 280 ℃ for 11 min. The temperature of the injection port was 250 ℃ and that of the transferline between GC and MSD was 280 ℃.

RESULTS AND DISCUSSION

Retention times of the drugs obtained from GC－NPD and GC－MSD analyses are shown in Tables 1 and 2. Characteristic ions of mass spectra of the compounds are shown in Table 2 also. Total ion current obtained from the analysis of the sample prepared as described in the derivatization procedure is shown in Figure 1. All the drugs yielded

Tab 1. GC－NPD data for TMS－TFA derivatives of narcotic analgesics and β－blockers

Compound	Retention time (min)	Relative retention time
Pethidine (PT)	3.079	0.4690
Anacin* (AA)	3.555	0.542
Trimeperidine (TR)	3.657	0.5570
Ethoheptazine (EH)	3.850	0.5864
Dextropropoxyphene (PP)	4.079	0.6213
	6.882	1.0482
Ethamivan (ET)	4.639	0.7066
Alprenolol (Alp)	4.948	0.7537
Oxprenolol (Oxp)	5.700	0.8682
Methadone (MT)	6.378	0.9715
Methaqualone (IS)	6.565	1.0000
Levorphanol (LF)	7.076	1.0778
Metoprolol (Met)	7.221	1.0999
Phenazocine (PE)	7.779	1.1849
Propranolol (Pro)	8.227	1.2547
Atenolol (Ate)	8.237	1.2547
Dihydrocodeine (DH)	9.106	1.3871
Sotalol (Sot)	9.235	1.4067
Nadolol (Nad)	10.025	1.5270
Codeine (CD)	10.163	1.5481
Dipipanone (DI)	10.358	1.5778
Ethylmorphine (EM)	10.492	1.5982
Morphine (MO)	10.751	1.6376
Heroin (HE)	12.852	1.9532
Acebutolol (Ace)	13.119	1.9983
Phenazocine (PH)	13.119	1.9983
Labetalol (Lab)	13.434	2.0463
	13.869	2.1126
Amfepramone (AN)	15.544	2.3677
Dextromoramide (MR)	16.214	2.4698
Nalbuphine (NA)	16.404	2.4987
Buprenorphine (BU)	19.389	2.9229
	26.079	3.9724

*: Stimulant

Tab 2. GC-MSD data for TMS-TFA derivatives of narcotic analgesics and β-blockers

Compound	MW	Peak number	Retention time (min)	Characteristic ion
Pethidine	247	2	4.942	71,172,247(M^+)
Anadol	261	33	5.260	172,261
Trimependine	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	284,129,427(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	14.390	73,236,429(M^-)
Heroin	369	23	16.390	327,369(M^+), 268
Acebutolol	336	24	16.516	284,129
Phenazocine	321	25	16.588	302,378
Labetalol	328	26	17.427	292
		27	17.827	292
Anileridine	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 first in the characteristic ions.

chromatographic peaks. As shown in Figure 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 by GC-MSD. The retention times of the drugs on GC-NPD were shorter than those on GC-MSD, because the column used in the GC-NPD was shorter.

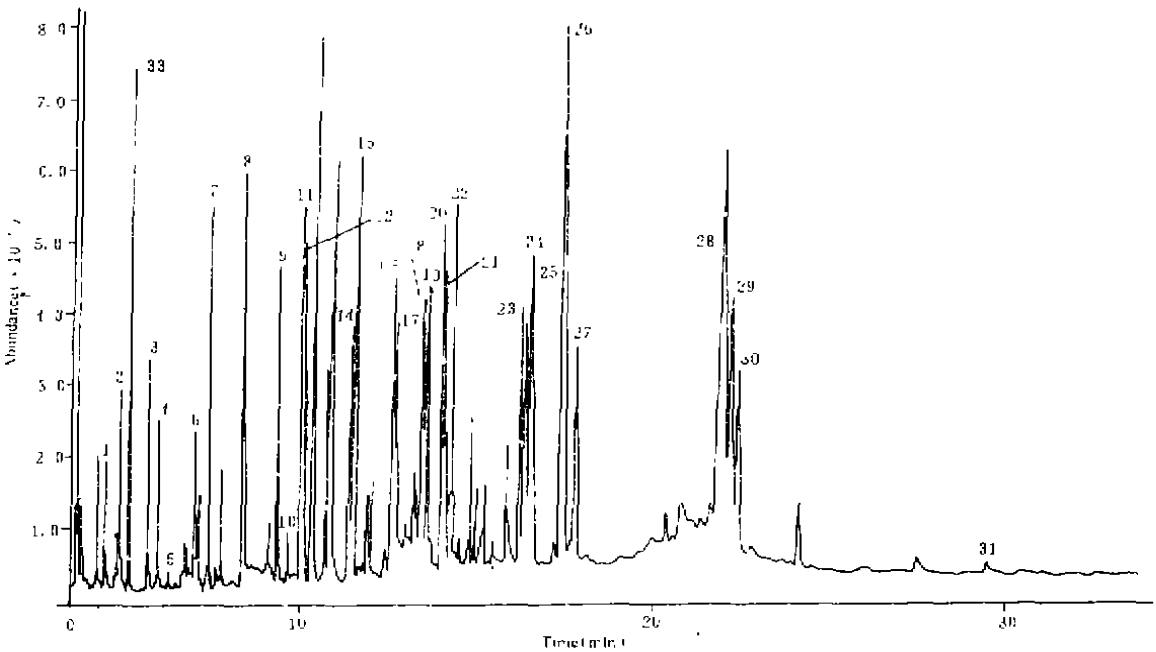

Fig. 1. Total ion current obtained from the GC – MSD analysis of a sample containing twenty – nine standards prepared as described in the derivatization procedure. The peak numbers in the figure are the same as those in Table 2.

Derivatization conditions

Derivatization reaction was affected by many factors such as the temperature of the reaction and the amounts of the derivatization reagents. In this procedure, MSTFA and MBTFA were used for derivatization. Different temperatures were tried for the reaction. It was found that four peaks were obtained from labetalol when 80 ℃ was used for heating after MSTFA and MBTFA were added, while two peaks were obtained if 70 ℃ was used instead of 80 ℃. The two peaks corresponded to the two isomers of labetalol. 70 ℃ was used in later experiments. For the derivatization of the mixture containing all the drugs as described in the sample preparation, 2 ml of MSTFA and 600 μl of MBTFA were used. It was found that for the derivatization of a sample containing three or four drugs or the extract of each urine sample, it was sufficient to use 100 μl of MSTFA and 30 μl of MBTFA.

Multiple chromatographic peaks

Most drugs yielded a single chromatographic peak under present derivatization conditions, however some drugs such as nalbuphine, methadone, sotalol and buprenorphine yielded multiple peaks. This might be caused by the side reactions or uncompleteness of the derivatization. Nalbuphine yielded two peaks at 13.229 and 16.404 min in GC – NPD and 17.427 and 22.511 min in GC – MSD. The chromatographic peak of longer retention time was used for the identification of the compound beacause M^+ ion (573) has been obtained in its mass spectrum. Two peaks were obtained from methadone in GC – NPD at

6.378 and 7.221 min and in 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 (9.235 and 9.643 min) and in GC－MSD (12.774 and 13.387 min). The first one was the main product of the derivatization, its mass spectrum showed ion of 344, the M^+ ion of sotalol－OTMS. Small peaks at 19.189 min in GC－NPD and 29.510 min in GC－MSD were obtained from buprenorphine followed by a high peak at 26.079 min in GC－NPD and 40.951 min in GC－MSD (not shown in Figure 1). The second peak was 17 times as high as that of the first one in GC－NPD and 3.3 times in GC－MSD. Both peaks were used for the identification of the compound.

Dextropropoxyphene

The decomposition of dextropropoxyphene was observed in GC－NPD and GC－MSD analyses. Two peaks were obtained from dextropropoxyphene 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 when the temperature of the injection port was decreased to 180 ℃, the height of the peak at 6.882 min in GC－NPD got much higher and corresponding to this peak, a new peak appeared at 9.973 min in GC－MSD. This result indicated that dextropropoxyphene might be decomposed at high temperature.

Internal standard

Phenazine was first tried as the internal standard, however the peak of phenazine overlapped that of pethidine. Methaqualone was then used. It was well separated from methadone and levorphanol as shown in Figure 1.

GC－NPD analysis described in this paper can be used for the screening of the drugs listed in Table 1 and GC－MSD be used for the confirmation of the results obtaind by GC－NPD. The presence of multiple chromatographic peaks from some compounds does not affect the detection of the drugs. This method has been used successfully for the detection of analgesics and β－blockers in the accreditation test organized by International Olympic Committee in November 1989.

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麻醉镇痛剂和 β－阻断剂类兴奋剂检测方法的研究

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提要 本文对兴奋剂的检测方法进行了研究。十九种麻醉镇痛剂, 九种 β－阻断剂, 一种刺激剂和一个内标物等共三十种药物可用配备有氮磷检测器和配备有质量选择检测器的气相色谱仪分别进行初筛和确证分析。

关键词 麻醉镇痛剂; β－阻断剂; 兴奋剂检测