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Note

High-speed liquid chromatography of alkaloids. I

ROBERT VERPOORTE and A. BAERHEIM SVENDSEN

Department of Pharmacognosy, University of Leyden, Gorlaeus Laboratories, Leyden (The Netherlands)

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High-speed liquid chromatography promises to be one of the most effective techniques ever devised for the separation of complex mixtures. Like gas–liquid chromatography, high-speed liquid chromatography is advantageous because of its high sensitivity and versatility. Compared with gas–liquid chromatography, the great advantage of high-speed liquid chromatography is that non-volatile and temperature-unstable compounds can also be submitted to analysis, such as many high-molecular-weight alkaloids. Recently, the high-speed liquid chromatography of some alkaloids has been reported, using ion exchange[1,2], liquid–liquid partition[3,4] or reversed-phase chromatography[5]. This paper reports the high-speed liquid chromatography of some alkaloids using liquid–solid chromatography.

TABLE I

UV MAXIMA

Alkaloid	*Solvent*	*Maxima (nm)*	$E^{1\%}_{1cm}$	*Wavelength of detection (nm)*
Quinine	Ethanol	236	1110	280
		278	133	
		332	163	
Quinidine	Ethanol	236	1110	280
		278	132	
		332	163	
Cinchonine	Ethanol	227	1214	280
		282	176	
		301	126	
		314	111	
		332	68	
Cinchonidine	0.1 *N* H_2SO_4	236	1874	280
		316	320	
Atropine	Methanol	252	6	254
		258	7	
		262	6	
Scopolamine	0.1 *N* H_2SO_4	251	12	254
		257	14	
		263	11	

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TABLE I *(continued)*

Alkaloid	*Solvent*	*Maxima (nm)*	$E^{1\%}_{1\,cm}$	*Wavelength of detection (nm)*
Cocaine	Ethanol	229	523	254
		274	33	
		281	30	
Strychnine	Ethanol	255	377	254
		280	128	
		290	102	
Brucine	Ethanol	264	260	254
		301	221	
Morphine	Ethanol	210	880	254
		236	176	
		287	56	
Codeine	Ethanol	212	840	254
		236	168	
		286	53	
Thebaine	0.1 *N* H_2SO_4	225	510	280
		285	253	
Heroin	Ethanol	230	171	280
		281	54	
Noscapine	Ethanol	291	100	280
		310	120	
Papaverine	Ethanol	240	2342	254
		280	234	
		315	148	
		327	148	
Narceine	Ethanol	278	22	280
Emetine	Ethanol	235	330	280
		281	131	
		360	3	
Cephaeline	Ethanol	235	330	280
		281	131	
		360	3	
Serpentine	Ethanol	252	888	254
		308	575	
		370	117	
Alstonine	Methanol	252	994	254
		309	658	
		336	70	
		369	116	
Reserpine	Methanol	216	924	254
		267	261	
		294	150	
Yohimbine	Methanol	226	1025	280
		280	214	
		291	178	
Raubasine	Methanol	227	1170	254
		280	197	
		292	175	
Tetrahydro-alstonine	Methanol	226	1240	254
		272	171	
		280	201	
		291	192	

EXPERIMENTAL

A Packard Model 8200 liquid chromatograph equipped with a 254 nm and a 280 nm UV detector was used. The column was a stainless-steel tube, 30 cm × 2 mm I.D., filled with Merckosorb Si 60 (5 μm). The balanced density slurry technique was used for filling the column. The column temperature was maintained at 20°. The flow-rates used were obtained with pressures of 50–250 kg/cm^2.

RESULTS AND DISCUSSION

It can be seen from Table I that there are big differences between the $E^{1\%}_{1\,cm}$ of the maxima of the alkaloids. The $E^{1\%}_{1\,cm}$ at the wavelength of detection is a measure for the sensitivity of detection. For the alkaloids investigated in the present work, the wavelength at which the highest sensitivity was found was determined, and the results are summarized in Table I. The sensitivity was tested for one alkaloid with a high $E^{1\%}_{1\,cm}$ (strychnine, $E^{1\%}_{1\,cm}$ = 377 at 254 nm) and one with a low $E^{1\%}_{1\,cm}$ (atropine,

TABLE II

RETENTION TIMES OF SOME ALKALOIDS

RT = retention time; FR = flow-rate.

Alkaloid	*Solvent system*											
	Chloroform–methanol						*Diethyl ether–methanol*					
	9 + 1		*8 + 2*		*7 + 3*		*8 + 2*		*7 + 3*		*6 + 4*	
	RT	*FR*	*RT*	*FR*	*RT*	*FR*	*RT*	*FR*	*RT*	*FR*	*RT*	*FR*
Quinine	–		6.4	0.81	5.9	0.75	3.9	0.85	2.7	1.43	2.2	1.34
Quinidine	–		6.5	0.81	6.2	0.75	3.7	0.85	2.9	1.43	2.4	1.34
Cinchonine	–		10.6	0.81	11.0	0.75	5.9	0.85	5.1	1.43	4.2	1.34
Cinchonidine	–		9.0	0.81	9.0	0.75	4.5	0.85	3.7	1.43	3.0	1.34
Atropine	–		–		–		–		9.4	1.38	7.4	1.20
Scopolamine	2.0	0.81	1.5	0.81	1.5	0.75	1.7	1.50	1.2	1.38	1.3	1.20
Cocaine	3.4	0.81	2.1	0.81	3.9	0.75	3.2	1.50	2.0	1.38	2.9	1.20
Strychnine	6.9	0.81	3.9	0.81	4.7	0.75	–		9.4	1.43	7.3	1.34
Brucine	6.8	0.81	3.9	0.81	4.6	0.75	–		17.2	1.43	13.1	1.34
Morphine	8.6	0.81	3.5	0.81	3.3	0.75	4.0	1.50	4.3	1.43	4.3	1.34
Codeine	4.6	0.81	2.7	0.81	2.9	0.75	3.4	1.50	3.6	1.43	3.4	1.34
Thebaine	4.1	0.81	2.7	0.81	3.0	0.75	2.7	1.50	3.7	1.43	3.7	1.34
Heroin	2.3	0.81	1.7	0.81	1.7	0.75	2.1	1.50	2.2	1.43	2.2	1.34
Narceine	–		7.5	0.81	5.7	0.75	–		–		8.5	1.34
Noscapine	1.1	0.81	1.0	0.81	1.1	0.75	0.6	1.50	0.6	1.43	0.7	1.34
Papaverine	1.1	0.81	1.1	0.81	1.2	0.75	0.8	1.50	0.8	1.43	0.8	1.34
Emetine	1.1	0.81	1.1	0.81	1.2	0.75	0.6	1.50	0.7	1.43	0.8	1.34
Cephaeline	–		5.9	0.81	8.6	0.75	–		–		8.1	1.34
Serpentine	–		–		–		–		–		–	
Alstonine	–		–		–		–		–		–	
Reserpine	2.2	0.38	1.0	0.81	1.1	0.75	2.9	0.33	3.0	0.33	2.2	0.40
Yohimbine	3.7	0.38	1.5	0.81	1.7	0.75	4.6	0.33	3.8	0.33	2.8	0.40
Raubasine	2.2	0.38	1.0	0.81	1.1	0.75	2.9	0.33	2.9	0.33	2.3	0.40
Tetrahydro-alstonine	2.1	0.38	1.0	0.81	1.1	0.75	2.5	0.33	2.7	0.33	2.2	0.40

$E^{1\%}_{1\,cm}$ = 6 at 254 nm). At the highest sensitivity setting on the apparatus, the amount that could be detected easily was *ca.* 25 ng for strychnine and 2 μg for atropine. Both results were obtained at a retention time of 2 min. In Table II, the retention times of the alkaloids obtained in six different solvent systems are summarized. Each retention time is the average of at least three analyses. As could be expected because of the weakly acidic properties of the silica gel used in the separation column, some tailing was observed for some of the alkaloids analyzed. It may, however, be possible to reduce the tailing by using basic solvent systems, as is often done in thin-layer chromatography. Some alkaloids were not eluted within reasonable times and these compounds are indicated by dashes in Table II. The number of theoretical plates of the column used was calculated to be 500–600.

REFERENCES

1 J. H. Knox and J. Jurand, *J. Chromatogr.*, 82 (1973) 398.
2 J. H. Knox and J. Jurand, *J. Chromatogr.*, 87 (1973) 95.
3 Cheng-Yi Wu and S. Siggia, *Anal. Chem.*, 44 (1972) 1499.
4 Cheng-Yi Wu, S. Siggia, T. Robinson and R. Waskiewcz, *Anal. Chim. Acta*, 63 (1973) 393.
5 G. H. Jolliffe and E. J. Shellard, *J. Chromatogr.*, 81 (1973) 150.