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Analysis of Some Multicomponent Drugs Using Milichrom Microcolumn Chromatographs

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Abstract—We analyze some two-, three-, and five-component drugs to demonstrate the potential of Milichrom microcolumn chromatographs. Chromatograph versions equipped with one syringe pump are suitable for analysis of complex mixtures in both isocratic and gradient modes. The procedures included in regulatory technical documents have been used for several years for quality control of drug manufacture.

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Milichrom microcolumn chromatographs have been manufactured by the Orel Research Instrument Plant for more than two decades. The Milichrom 4 and Milichrom 5 versions have been used most widely. Their convenience of use is due to complete automation, easy operation, relatively low requirements for water and organic solvent quality, and moderate water and solvent consumption. The Milichrom 5-3 and Milichrom 6 versions have been manufactured in recent years. Their primary distinction from the prior art instruments is two syringe pumps instead of one. These chromatographs in their characteristics approach the current level for chromatographs. The Russian literature is rich in the description of analysis of multicomponent drugs on Milichrom chromatographs; see, e.g., [1–7]. Table 1 lists selected characteristics of these analyses. In our opinion, even this comparatively small selection reflects some tendencies in the use of Milichrom chromatographs. Most research concerns reversed-phase HPLC [1–5, 7]; descriptions of normal-phase chromatography are far rarer [6]. Most of these separations are in the isocratic mode [1–6]; gradient elution is applied relatively rarely [7]. While the first of the aforementioned tendencies is in general characteristic of the use of HPLC, the second is due to the specifics of Milichrom chromatographs: gradient elution on them is difficult. However, the experience of the authors of this work demonstrates that these instruments can succeed in the separation and quantitative analysis of complex mixtures both in isocratic and gradient modes. This work reports some results of our research.

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EXPERIMENTAL

Reagents. Acetonitrile for chromatography (Cryochrom) and bidistilled water were used to prepare mobile phases and dissolve references and test drug samples. The references used were pharmaceuticals verified by quality control at the manufacturing enterprise for conformity to the requirements of regulatory documents. The other reagents used were of at least pure for analysis grade.

Instruments. Milichrom 4 and Milichrom 5 were used. The columns used 80 × 2.0 mm in size were packed with reversed-phase sorbents Separon SGX C18 and Nucleosil 100 C18 with a particle size of 7.0 μm. pH in the buffer solutions used to prepare mobile phases was monitored with a pH-673M ionometer/millivoltmeter equipped with a glass indicator electrode and an Ag/AgCl reference electrode.

For selected drugs, the solution preparation protocol for analysis is described in Table 2.

All solutions were filtered through hydrophilic membrane filters with 0.45 μm pore sizes; Teflon filters stable in water–acetonitrile solutions are preferred for this purpose.

Analysis. A test solution and a reference solution were chromatographed. The mobile phase flow rate was 0.15 mL/min. The volume of the injected sample was 5.0 μL. The mobile phase composition is specified in Table 3.

RESULTS AND DISCUSSION

Common features of chromatographic separation on Milichrom chromatographs. The main distinction of chromatography on Milichrom chromatographs is that this is a batch process. While the mobile phase flows continuously through a conventional

Table 1. Characteristics of some drug analyses on Milichrom chromatographs

Drug, substances	Column	Mobile phase and its flow rate	Wavelength, nm	Source
Local anesthetics: Novocaine, Anesthesin, Papaverine hydrochloride	64 × 2.0 mm Separon C18	CH_3CN : PB, pH 2.5, 0.1 mL/min 60 : 40 80 : 20	290 290; 260	[1]
Corticosteroids in ointments: Prednisolone, Sinaflan, Hydrocortisone	64 × 2.0 mm Separon C18	C_2H_5OH, 0.05 mL/min	240	[2]
Aubergine tablets: 2-bromo-α-ergocryptine mesylate, 2-bromo-β-ergocryptine mesylate	64 × 2.0 mm Silasorb C18 (5.0 μm)	THF : 0.01 M $(NH_4)_2HPO_4$ = 47 : 53, 0.03 mL/min	254	[3]
Oral contraceptives: Ethinylestradiol, Levonorgestrel	120 × 2.0 mm SGX-RP-S-ODS (8.0 μm)	CH_3CN : H_2O = 3 : 2, 0.15 mL/min	220	[4]
Multivitamins: retinol acetate, ergocalciferol, α-tocoferol acetate	120 × 2.0 mm Separon SGX (5.0 μm)	CH_3OH : H_2O = 99 : 1	326; 264; 286	[5]
Oil vitamin solutions: Vitamins A, E, K3	64 × 2.0 mm Silasorb 600 (5.0 μm)	Hexane : diethyl ether = 6 : 94	220; 320	[6]
Multicomponent drug formulation: water-soluble vitamins, calcium pantothenate		CH_3OH : PB, pH 7.0 stepwise gradient	210 260	[7]

Note: PB stands for phosphate buffer; THF, for tetrahydrofuran.

Table 2. Solution preparation protocol for analysis of some drugs on Milichrom chromatographs

Drug	Analyte	Sample weight, g (volume, mL)	Solvent (100 mL)
Ascofen tablets	Paracetamol, Caffeine, Aspirin	0.125	CH_3CN : H_2O : CH_3COOH = 30 : 67 : 3
Pentalgin ICN tablets	Paracetamol, Analgin, Caffeine, Codeine phosphate, Phenobarbital	0.160	0.120 g Na_2SO_3 in CH_3CN : H_2O = 15 : 85
Neo-theophedrine tablets	Paracetamol, Theophylline, Caffeine, Ephedrine hydrochloride, Phenobarbital	0.160	CH_3CN : (0.025 M K_2HPO_4; pH 3.0) = 15 : 85
Prochodol Forte tablets	Paracetamol, Caffeine, Codeine phosphate	0.160	CH_3CN : 0.025 M K_2HPO_4 = 1 : 9
Codelac Phyto syrup	Codeine phosphate, Nipagin, Nipasol	(5.0)	CH_3CN : H_2O = 3 : 7
Clarisens syrup	Loratadine, Nipagin, Nipasol	(5.0)	CH_3CN : H_2O = 3 : 7
Co-trimoxazole suspension	Trimethoprim, Sulfamethoxazole, Nipagin, Nipasol	2.5	CH_3CN : H_2O = 1 : 4

instrument, through a Milichrom it flows only during analysis. This drawback deteriorates the analysis precision and accuracy. To compensate in part this negative effect, the instruments are equipped with a regeneration facility; that is, the column was purged with the mobile phase for 1–2 min before analysis, after which the test sample was injected. In addition, 5–10 μL of buffered volumes is syringed before and after sampling. This reduces the interference from sample injection. Gradient separation has some specifics because of the design of Milichrom instruments. In the isocratic mode, the position of the syringe pump is unimportant. For gradi-

Table 3. Results of HPLC analysis of some multicomponent drugs ($n = 9$; $P = 0.95$)

Drug	Analyte	Norm in regulatory documents, g/tablet (g/5 mL)	X_{av}	s	$s_{X_{av}}$	ε, %
Ascofen tablets	Paracetamol	0.190–0.210	0.19800	0.00150	0.00050	0.60
	Caffeine	0.037–0.043	0.00395	0.00005	0.00002	1.00
	Aspirin	0.190–0.210	0.20140	0.00210	0.00070	0.82
Pentalgin ICN tablets	Paracetamol	0.285–0.315	0.30210	0.00340	0.00113	0.89
	Analgin	0.285–0.315	0.29640	0.00260	0.00087	0.69
	Caffeine	0.04625–0.05375	0.50120	0.00630	0.00210	0.99
	Codeine phosphate	0.0072–0.0088	0.00789	0.00009	0.00003	0.90
	Phenobarbital	0.009–0.011	0.09760	0.00180	0.00060	1.45
Neo-theophedrine tablets	Paracetamol	0.285–0.315	0.30230	0.00230	0.00077	0.60
	Theophylline	0.0925–0.1075	0.10120	0.00200	0.00067	1.55
	Caffeine	0.04625–0.05375	0.05065	0.00070	0.00023	1.09
	Ephedrine hydrochloride	0.0185–0.0215	0.01940	0.00027	0.00009	1.09
	Phenobarbital	0.009–0.011	0.00986	0.00011	0.00004	0.88
Prochodol forte tablets	Paracetamol	0.475–0.525	0.50340	0.00640	0.00213	1.00
	Caffeine	0.04625–0.05375	0.05004	0.00064	0.00021	1.01
	Codeine phosphate	0.0072–0.0088	0.00786	0.00008	0.00003	0.80
Codelac phyto syrup	Codeine phosphate	(0.004–0.005)	0.00475	0.00006	0.00002	0.99
	Nipagin	(0.0034–0.0041)	0.00370	0.00002	0.00001	0.43
	Nipasol	(0.0011–0.0014)	0.00120	0.00002	0.00001	0.98
Clarisens syrup	Loratadine	(0.0045–0.0055)	0.00486	0.00006	0.00002	0.97
	Nipagin	(0.0013–0.0020)	0.00165	0.00002	0.00001	0.95
	Nipasol	(0.00065–0.00100)	0.00084	0.00001	0.00000	0.94
Co-trimoxazole suspension	Trimethoprim	(0.036–0.044)	0.03950	0.00040	0.00013	0.80
	Sulfamethoxazole	(0.180–0.220)	0.20250	0.00340	0.00113	1.32
	Nipagin	(0.0012–0.0018)	0.00155	0.00002	0.00001	1.02
	Nipasol	(0.000375–0.000675)	0.00050	0.00001	0.00000	1.59

ent elution, the pump must be positioned so that the spindle moved from top down to replace the mobile phase. This is because of the fact that all steps of the mobile phase are syringed by the pump before analysis; the steps with higher percentages of the organic modifier, which have lower densities, are syringed first. These steps should have the uppermost position in the injector of the mump, which slows down solvent stirring. If this rule is violated, gradient elution with such an instrument is practically impossible.

Results of analysis. Figures 1 and 2 exemplify elution curves obtained in analysis of some multicomponent drugs on a Milichrom 5 chromatograph. The separation parameters are indicated in the legends. Both isocratic and gradient elutions resulted in clear separation and satisfactory peak shapes. Our developed processes

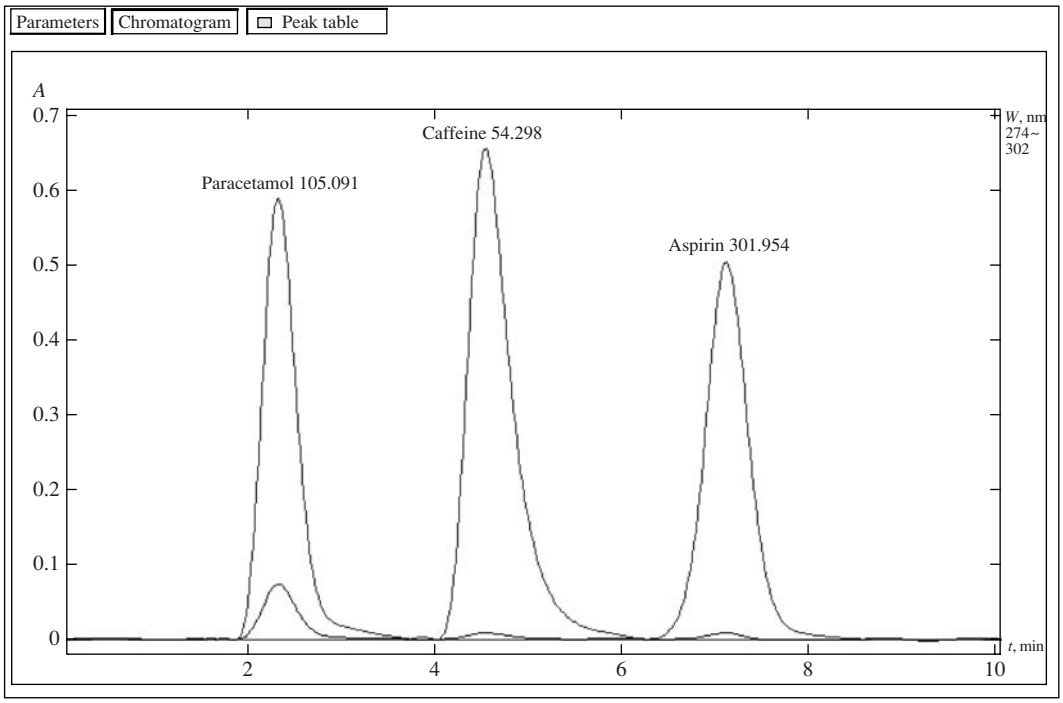

Fig. 1. Elution curve for a test solution of Ascofen tablets. Column: 2.0 × 80 mm, Nucleosil 100 C18 with the particle size 7.0 μm. Mobile phase: $CH_3CN : H_2O : CH_3COOH = 30 : 67 : 3$. Detection at 220 nm.

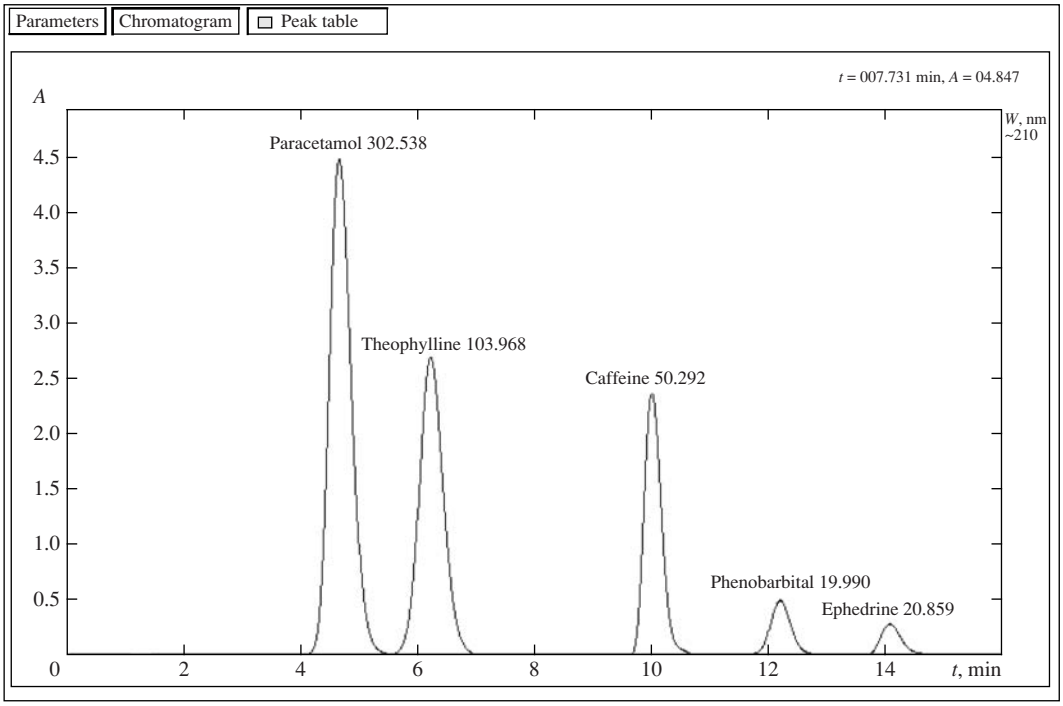

Fig. 2. Elution curve for a test solution in analysis of Neo-theophedrine tablets. Column: 2.0 × 80 mm, Separon SGX C18 with the particle size 7.0 μm. Mobile phase: stepped gradient: $CH_3CN : H_2O = 1 : 9$ (700 μL–$CH_3CN : H_2O = 3 : 7$ (800 μL)–CH_3CN : 0.025 M $KH_2PO_4 = 3 : 2$ (800 μL). Detection at 207 nm.

were used for monitoring pilot samples manufactured in the laboratory in the course of technology development. Our results conform to technological loads (Table 3).

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