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Prof. Dr. B. Unterhalt
Institut für Pharmazeutische Chemie
Hittorfstr. 58–62
D-48149 Münster

Pharmaceutical Development, Jenapharm GmbH & Co. KG, Jena, Germany

Determination of testosterone, testosterone propionate, testosterone enanthate, testosterone undecanoate in pharmaceutical preparations by HPLC

S. CLAUSSEN, M. BÖSE and M. DITTGEN

The use of testosterone esters as pro-drugs has been described by several authors [1–4]. Several papers presented HPLC procedures for the determination of testosterone and testosterone esters in human plasma and pharmaceutical formulations [5–8]. Usually, porous silica gel columns with reversed phase C_{18} were used in those procedures and in most cases a $UV_{243\,nm}$ detector was used. There are, however, no publications to determine testosterone (T), testosterone propionate (TP), testosterone enanthate (TE) and testosterone undecanoate (TUD) in pharmaceuticals with fast LC.

In this work, we developed a precise and reliable method based on UV detection for the baseline separation of T, TP, TE, TUD in not more than 10 min.

With this tecnique, quite a good separation of testosterone and testosterone esters was obtained (Fig.). The method was optimised and proofed for robustness using a factorial design approach (Table).

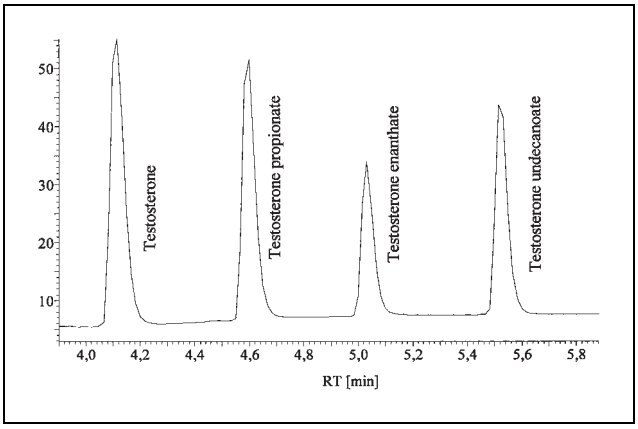

Fig.: Separation of (T), (TP), TE and TUD by the gradient HPLC on Micra RP 18

Experimental

1. Materials and agents

T and TP were obtained from Fluka Chemie Ag, CH-Buchs, TE from AK 20 Nobel and TUD from Jenapharm GmbH & Co. KG. HPLC acetonitrile was obtained from E. Merck, Darmstadt. Purified water was used.

2. Instrumentation and procedure

The chromatography was performed using a Shimadzu LC 10A chromatograph. The liquid chromatograph was equipped with a LC-10 AS pump system and a variable-wavelength UV-detector. Separations were performed on a 33 × 4.6 mm i.d. 1.5 μm Micra RP 18 column (Bischoff chromatography, Leonberg, FRG). The gradient system consisted of acetonitrile/water with following time procedure: 0–2 min 10% acetonitrile in water, 2–5 min linear up to 96% acetonitrile, 5–6 min 96% acetonitrile at a flow-rate of 1.4 ml/min at 40 °C ± 0.3 °C. The detector wavelength ewas 243 nm. In all cases the injection volume was 10 μl in duplicate.

The concentrations of testosterone and testosterone esters were calculated by peak area ratio of analyte in comparison with calibration curves which were linear in the described working ranges. An overall chromatographic time of 6 min provides an efficient method. The analysis time is reported

Table: Factorial design set (a) and results (b); established working range (c) for the method

a) 2^4cube + star Statistical design for optimissing of chromatographic conditions

Set	−x	−1	0	+1	+x
Variable					
Start acetonitrile (%)	6	8	10	12	14
End acetonitrile (%)	92	94	96	98	100
Flow-rate (ml/min)	0.6	0.8	1.0	1.2	1.4
Temperature (°C)	30	35	40	45	50

b) Results of selected experiments

Exp. no.	Res* T-TP	Res* TP-TE	Res* TE-TUD	Sym T	Sym TP	Sym Te	Sym TUD
1	5.516	6.108	7.193	1.269	1.247	1.328	1.097
11	5.73	5.093	6.189	1.379	1.284	1.229	1.211
12	4.902	5.638	6.844	1.391	1.373	1.349	1.306
13	6.825	5.328	5.808	1.140	1.272	1.163	1.101
26	6.178	4.779	5.16	1.184	1.31	1.128	1.174

Res* − Resolution
Sym − Symmetry
Boldface-optimum

c) working range (W) of the method, retention time (R), Slope (S) and correlation coefficient (C) for the calibration curve

Substance	R (min)	W (ng/10 µl injection)	S (µV · s/µg/ml)	C
T	4.10	0.1012–0.6072	7061	0.9997
TP	4.59	0.1016–0.6096	5382	0.9997
TE	5.02	0.1042–0.6252	2091	0.9991
TUD	5.53	0.1004–0.6024	1098	0.9974

in the literature [5, 6]. Short reequilibration time of the column and a variable rinse time for sample clean-up with 10% acetonitrile in water prefers the method for build-in in automated analysis systems.

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Sven Claußen
Pharmaceutical Development
Jenapharm GmbH & Co. KG
Otto-Schott-Str. 15
D-07745 Jena

Faculty of Pharmacy, Medical University, Sofia, Bulgaria

Development of biodegradable poly(α-methylmalate) microspheres

K. YONCHEVA and N. LAMBOV

During the recent years some novel biodegradable oligoesters based on tartaric, malic and other acids have been used for the development of biodegradable drug delivery systems apart from conventional poly(lactic acid) (PLA), poly(D,L-lactide-co-glycolide) (PLCA), polycaprolactone (PCL), and their co-polymers [1]. Some of our previous papers reported the possibilities to prepare chemically crosslinked drug delivery systems based on malic acid via polycondensation [2]. Being biocompatible and biodegradable is the main advantage of the polymalates as drug carriers [3]. Their degradation results in malic acid which is a natural metabolite in the Crebs Cycle. Thus Belcheva et al. [4] implemented a polycondensation method to prepare a series of polymalates of Mw > 5000 with potential application as biodegradable drug carriers. Wada et al. [5] used low molecular weight PLA oligomers for achieving drug release rates higher than those provided by high molecular weight PLA.

The above mentioned studies provoked our investigations aiming at the preparation of drug loaded microspheres based on a novel biodegradable poly(α-methyl-malate) polymer (PMM). Microspheres were prepared by a simple solvent evaporation method. Comparatively, microspheres from traditionally used PLGA co-polymer were obtained under the same process conditions. Isopropylantipyrine was selected as a model agent for incorporation because it is a lowly soluble compound and release could be followed easily by spectrophotometry.

PMM microspheres were obtained with a drug loading of 30% and PLGA microspheres were loaded with 28% drug. A negligible amount of drug was wasted in the external aqueous phase since the drug is slightly soluble in water. Scanning electron microscopy (SEM) showed that both samples of microspheres have spherical shape and smooth surfaces. No drug crystals are observed on the microsphere surfaces. Microspheres obtained from PMM seem to be on average smaller (5–80 µm) than those obtained from PLGA (30–80 µm). The reason for this difference might be the higher viscosity of the inner organic phase of the PLGA emulsion. This leads to the formation of larger drops during emulsification and respectively to

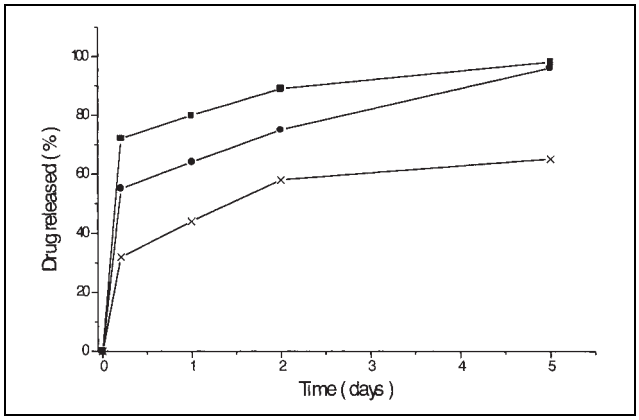

Fig. 1: In vitro release of isopropylantipyrine from: lyophilized PMM microspheres (■); non-lyophilized PMM microspheres (●); and PLGA-microspheres (×)