ANALYTICAL METHODS USED FOR BIOPHARMACEUTICAL INVESTIGATIONS

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Therapeutic effect of drugs depends not only on chemical structure of the active compound and its purity, but on many other physical and chemical factors connected with the form of drug as well. It depends also on pathophysiological factors of the living organism. Very different results of biological availability test are proved for the same active compound contained in different drug forms. Moreover, these differences were proved for the same drug form manufactured by other ways and different conditions of living organism, used for testing.

The realizing of these questions has changed opinion of a technological proceeding and analytical methods of modern drug investigation. Two problems are discerned in biopharmaceutical investigations:

1. Pharmaceutical release test.
   It concerns the releasing time of the active compound from drug form and it is determined by "in vitro" methods.
2. Biological availability test.
   It is the pharmaco-kinetical problem in mathematical conception and concerns the absorption, distribution and elimination of active substance in living organism.

There are a lot of instruments designed either for determination of releasing or of absorption time but it is not the subject of this report. In this report, attention will be paid to the fact that a good method of assay of active compounds is the main base in bio-pharmaceutical investigations. These methods will be the subject of the report.

Each method will be discussed with regard to its availability in investigation either "in vitro" or "in vivo", because in both cases requirements are different. Two tables have been made for better transmission to the readers of the estimation of each of these methods and their main properties. The methods were marked out on the tables according to their availability and frequency of application.

It was made with references to a lot of publications but final estimate is subjective and open to question.

The General Properties and Requirements for Methods

I. Specification of the substance being determined

It is very important in all cases that auxiliary substances used in the drug form like filling substances for tablets, bases of ointments and buffers or organism's fluids and metabolites should not influence determination. As a matter of fact there is no ideal method fulfilling this condition.

II. Sensitivity

This property is not very important in "in vitro" investigations because the quantity used in the tests can be increased. Some difficulties can arise in cases of low sensitivity when the concentration must be small. It can arise at the end of a determination or in a case of a slightly soluble substance. Sensitivity of a method "in vivo" is very important. Low concentrations are employed which are changeable at different stages of the determination. The quantity of a solution is usually limited. Very often compounds are determined indirectly by means of metabolite's determination. Concentration of investigated substance ought to be 1/10 to 1/20 of average value at the point of maximum concentration. The importance of sensitivity in biopharmaceutical analysis is shown in the following examples: after application to a man of 10mg. of Nitrozepam, maximum concentration in plasma is 100*ug*. per 1 cm^3, but after application to a man of 20 mg. of Chlordiazepoxid (Librium) maximum concentration in urine is 1 *ug*. per 1cm^3 only. This determination requires a very sensitive method.

III. Speed of Determination

It is usually not important in "in vitro" but it is a most frequent and important condition in "in vivo" investigations because of the little stability of substances being determined. In organism's fluids there occurs enzymatic decomposition, binding with proteins and adsorption onto glass; the longer the time of test, the more the effects.

IV. Possibility of Automation

It is important in either "in vitro" or "in vivo" investigation. Automation is possible mainly with application of spectrophotometry, gas chromatography, conductometry and isotope methods.

V Sensitivity of Instruments

Is especially very important in "in vivo" investigations. The same concerns **purity of reagents.**

VI. Route of drug administration

Influences choice of analytical method. By oral application you can expect a large and fast chemical decomposition of the substance and then determination of metabolites can be carried out.

TABLE 1

The general properties and requirements for methods

Property of Method	In Vitro	In Vivo
Specification	xxx	xxx
Sensitivity	x	xxx
Speed	x	xxx
Possibility of automation	xx	xx
Sensitivity of instruments	xx	xxx
Purity of reagents	xx	xxx
Route of drug administration		xxx

– not important or useless
x slight important or slight useful
xx important or useful
xxx very important or very useful.

TABLE 2

Evaluation of Methods

Methods	*in vitro*	*in vivo*
Titration	X	–
Ultra violet spectrophotometry	XXX	XX
Colorimetry	XX	X
Fluorescence spectrophotometry	XX	XXX
Column chromatography	XX	XX
Thin-layer chromatography	XX	XX
Gas and liquid chromatography	XX	XXX
Isotopic method	XX	XXX
Microbiological methods	X	XXX
Polarography and Oscillopolarography	X	X

From this short report, it can be seen that pharmaceutical analytical laboratories will have to change their bases of drugs evaluation. It will no longer be sufficient to carry out only disintegration time of solid drug forms and determination of total contents of active compounds in these ones.

It is difficult to imagine that in future any drug will be able to be registered without even pharmaceutical release test. The duty of pharmaceutical industry will have to increase. Apart of fundamental chemical, pharmacological and clinic documentation, it will be required to submit data of absorption and elimination of each form of drug separately. According to the above, pharmaceutical laboratories must be supplied with modern very sensitive instruments and more staff will have to be trained to handle these problems.

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