# VARIOUS APPLICATIONS OF HPLC: SPECIFICALLY QUANTITATIVE ANALYSIS

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ABSTRACT: HPLC (High Performance Liquid Chromatography) is widely used technique for analysis of biological samples. In this article some papers where the HPLC is used as a technique for analysis of blood are reviewed .HPLC is used in quantitative determination of acetone in blood sample, ofloxacin in human blood plasma using SPE, tocopherol in plasma and cellular elements of the blood. Some papers are reviewed and how HPLC techniques applicable to biological samples are studied.

Keywords: HPLC, acetone, Blood sample

**Introduction:** HPLC is liquid -liquid chromatographic method. Separation of components of sample takes place due to partition of the solute between the liquid stationary phase and the mobile phase. Liquid stationary phase is supported on solid inert support. Liquid mobile phase travels over stationary liquid phase under pressure, hence the technique is known as High Performance Liquid chromatography. HPLC is used in clinical test of urine , blood[1] , in detection of phenolic compounds and pollutants in water[2] ,in analysis of antibiotics ,sedatives, steroids [3],analysis of textile dyes, drugs[4],and analysis of food preservatives[5]

**T**he quantitative determination of acetone in human blood by using high-performance liquid chromatography (HPLC) is reported in literature [1]Acetone is colorless, flammable volatile solvent it is the simplest ketone the other name for acetone are propanone or dimethyl ketone. The chemical formula of acetone is $(CH_3)_2CO$.Acetone is not considered harmful to human beings. World Health Organization has classified acetone as non-carcinogenic. Acetone in saliva by liquid chromatography with fluorescence can be determined.

**Methods and materials:**

**For quantitative analysis of biological samples by HPLC various methods are used:**

**Akgul Kalkan et.al . (2016). Performed Quantitative Clinical Diagnostic Analysis of Acetone in Human Blood by HPLC u**sing 2,4-DNPH (2,4-dinitrophenylhydrazone ) as derivatizing agent in HPLC elution and recovery problems were solved .The retention time ($t_R$) was obtained as 3.80 min. The column used was thermoAcclain C18 for acetone as the derivative 2,4 – dinitrophenylhydrazone with retention time 12.10 min and flow rate 1 ml/min. The parameters of method validation are: method is linear in the range 0.5 to 20 $mmolL^{-1}$,the regression equation and correlation coefficient were obtained as $y = 0.7361x + 0.0877$ with a 0.9967, correlation coefficient ($R$).The detection limit and limit of quantification were found as 0.041 and 0.136 $mmolL^{-1}$ respectively. The recovery was found to be 98%

The quantitative determination of acetone in human blood was carried ou by this method .in this UV-Vis DAD (diode array detector) detector was used with HPLC.this method is rapid, economic, sensitive and selective.

The liquid chromatography method combining with solid phase dispersion for detecting phenolic compounds in olive oil samples was used by Monasterio RP et.al. In this method 0.5g of olive oil, sorbent used was 1.0g of Florisil and eluting solvent 1 ml methanol-water. The detection limit and limit of quantification was found in the range of 0.02-0.75 and 0.08-2.50 mg kg-1 respectively .The RSD were ranged between 2.1% and 14.8%.Recovery rate was74.8% to 95.0%[6]

**Joshi, Shalini** cited different mobile phases, column used for characterising antimicrobial activities for various classes of anytibiotics [3]

**Lynda J. Hatam and Herbert J. Kayden** determined tocopherol in plasma and cellular elements of the blood by HPLC method by which the volume of blood sample required is reduced[7]

**Fernandez P** et al used the method HPLC with UV detector ,eluent used was methanol (pH 7 )-phosphate buffer .The flow rate gradient(time, flow rate ) used was 0.0 min. 0.4 mL/min,6.0 min. 0.7 mL/min; 8.0 min, 1.0 mL/min, 10.0 min, 0.7 mL/min; 11.0 min, 0.6 mL/min; 12.0 min,0.4ml/min .The working pressure ranged from 500 to 2,300psi.The injected volume was 25ul .The recovery rate for cocaine was 87.2% from plasma and 96.5% from urine and for BZE 76.9% from urine and 82.7% from plasma .[8]Mustarichie, Resmi et al carried out oflaxacin study in human blood plasma and later validity of method using HPLC SPE UV detector.

## Conclusion

From the above review study it has been observed that the HPLC method was used for analysis of biological samples where accuracy, recovery rate was found to be good.

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